BD FACSAria
User's Guide

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FCC Information

For Research Use Only. Not for use in diagnostic procedures.

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense.

Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Réglement sur the matériel brouilleur du Canada.

History

Revision	Date	Change Made
333608	1/03	Initial release
338645	10/04	Updated for new instrument and software features: bubble and ND filters, adjustable nozzle, waste cap with baffle and disposable filtered cap, adjustable nozzle, running samples using BD FACSDiva software version 4.1
640760	5/06	Updated for BD FACSDiva software version 5.0 and new ceramic nozzles

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About This Guide

This user's guide contains the instructions necessary to operate and maintain your BD FACSAria™ flow cytometer. Because many instrument functions are controlled by BD FACSDiva™ software, this guide also contains basic software information needed for instrument setup. To familiarize yourself with the software, do the tutorials in the BD FACSDiva software *Getting Started* guide. For detailed information on software features, refer to the *BD FACSDiva Software Reference Manual*.

The *BD FACSAria User's Guide* assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, refer to the documentation provided with your computer.

New users of the BD FACSAria flow cytometer should read:

- Chapter 1 to become familiar with instrument components
- Chapter 2 to understand how the instrument works and to learn about the software components used to control different subsystems
- Chapter 3 to see where software components are located

Instructions for routine acquisition, analysis, and sorting can be found in Chapters 4 and 5.

Once you become familiar with routine operation and need only a quick reminder of the main steps, use the quick reference guide provided with this user's guide.

Conventions

The following tables list conventions used throughout this guide. Table 1 lists the symbols that are used in this guide or on safety labels to alert you to a potential hazard. Text and keyboard conventions are shown in Table 2

Table 1 Hazard symbols[a]

Symbol	Meaning
	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death
	Electrical danger
	Laser radiation
	Biological risk

a. Although these symbols appear in color on the instrument, they are in black and white throughout this user's guide; their meaning remains unchanged.

Table 2 Text and keyboard conventions

Convention	Use
☑ **Tip**	Highlights features or hints that can save time and prevent difficulties
NOTICE	Describes important features or instructions
Italics	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, "choose File > Print" means to choose Print from the File menu.
Ctrl-X	When used with key names, a dash means to press two keys simultaneously. For example, Ctrl-P means to hold down the Control key while pressing the letter *p*.

Technical Assistance

For technical questions or assistance in solving a problem:

- Read the section of the user's guide specific to the operation you are performing.
- See Chapter 7, Troubleshooting.

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.

When contacting BD Biosciences, have the following information available:

- product name, part number, and serial number
- any error messages
- details of recent system performance

For instrument support from within the US, call (877) 232-8995.

For support from within Canada, call (888) 259-0187.

Customers outside the US and Canada, contact your local BD representative or distributor.

Safety and Limitations

The BD FACSAria™ flow cytometer is equipped with safety features for your protection. Operate only as directed in the user's guide. Do not perform instrument maintenance or service except as specifically stated. Keep this safety information available for reference.

Laser Safety

Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. The main hazard of laser radiation is direct or indirect exposure of the eye to thermal radiation from the visible and near-infrared spectral regions (325–1,400 nm). Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness.

There are other potentially serious hazards in other spectral regions. For cytometers that contain UV lasers, excessive ultraviolet exposure produces an intolerance to light (photophobia) accompanied by redness, a tearing discharge from the mucous membrane lining the inner surface of the eyelid (conjunctiva), shedding of the corneal cell layer surface (exfoliation), and stromal haze. These symptoms are associated with photokeratitis, otherwise known as snow blindness or welder's flash, which results from radiant energy–induced damage to the outer epidermal cell layer of the cornea. These effects can be the result of laser exposure lasting only a fraction of a second.

Laser Product Classification

Laser hazard levels depend on laser energy content and the wavelengths used. A numbered system is used to categorize laser products according to different hazard levels. The higher the classification number, the greater the potential hazard. The BD FACSAria flow cytometer is a Class I (1) laser product per 21 CFR Subchapter J and IEC/EN 60825-1:1994 + A2:2001. The lasers and the laser energy are fully contained within the instrument structure and call for no special work area safety requirements except during service procedures. These procedures are to be carried out only by BD Biosciences service personnel.

Precautions for Safe Operation

Modification or removal of the optics covers or laser shielding could result in exposure to hazardous laser radiation. To prevent irreparable damage to human skin and eyes, do not remove the optics covers or laser shielding, adjust controls, or attempt to service the instrument any place where laser warning labels are attached (see Precaution Labels on page xviii).

Use of controls or adjustments or performance of procedures other than those specified in the user's guide may result in hazardous radiation exposure.

Keep all instrument doors closed during instrument operation. When operated under these conditions, the instrument poses no danger of exposure to hazardous laser radiation.

Electrical Safety

Lethal electrical hazards are present in some lasers, particularly in laser power supplies. Many portions of the electrical system, including the printed circuit boards, are at a dangerous voltage level. To prevent shock injury, follow these guidelines.

- **Turn off the power switch and unplug the power cord** before servicing the instrument, unless otherwise noted.
- Connect the equipment only to an approved power source. Do not use extension cords. If cords, plugs, or cables are damaged, immediately contact BD Biosciences for a replacement.
- Do not remove the grounding prong from the power plug. Have a qualified electrician replace any ungrounded receptacles with properly grounded receptacles in accordance with the local electrical code.
- For installation outside the US, use the proper power cord. Contact your local BD representative for more information.

Do not plug the fluidics cart power cord into a wall outlet. Plug the cord into the cytometer only. This ensures proper electrical grounding and protects against electrical shock or damage to the instrument.

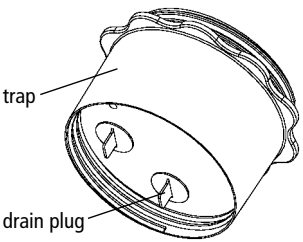
A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the voltage warning light is illuminated, or when the software indicates that the plate voltage is on. The plates remain energized even when the sort block door is opened.

The barb and the stream-charging wire conduct electrical charge during sorting. To prevent shock, do not touch the barb or the stream-charging wire when the voltage warning light is illuminated or any time during sorting.

Biological Safety

All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, follow these guidelines.

- Handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.
- Expose waste container contents to bleach (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precaution and wear suitable protective clothing, eyewear, and gloves.
- Prevent waste overflow by emptying the waste container daily and whenever the waste indicator shows the waste is getting full.
- The waste tank can become pressurized when the cytometer is running. Always disconnect the tank from the wet cart before you empty it. Wait at least 1 minute for pressure to dissipate before you remove the waste cap or sensor.
- Replace the waste tank cap every month. Failure to do so could cause the waste tank to malfunction. For tracking, write the date on the waste cap label each time you change the cap.
- Do not wet the waste tank cap. If wet, the filter in the cap will cause the tank to malfunction. To keep the cap dry, place it on the bench label-side up when it is not on the tank. If you see liquid in the waste cap trap, remove the drain plug and fully drain the liquid before you replace the plug.



Cell sorters that use droplet generation methods, like the BD FACSAria, can produce aerosols around the sample stream. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door closed during sorting. If you need to access the sort block, turn off the stream before opening the door.

For information on laboratory safety, refer to the following guidelines. NCCLS documents can be ordered online at www.nccls.org.

- Schmid I, Nicholson JKA, Giorgi JV, et al. Biosafety guidelines for sorting of unfixed cells. *Cytometry.* 1997;28:99-117.

- *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline-Second Edition.* Wayne, PA: National Committee for Clinical Laboratory Standards, 2001. NCCLS document M29-A2.

- *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline-Third Edition*. Wayne, PA: National Committee for Clinical Laboratory Standards; 2004. NCCLS document H18-A3.

General Safety

Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, follow these precautions.

- Keep your hands and clothing away from the sample injection chamber when a tube is loading or unloading. Do not place objects underneath the sample injection chamber.

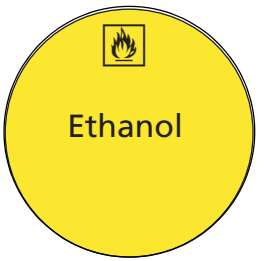
- Keep your hands away from the sort aspirator drawer during sorting. The drawer can close at any time without warning if a clog is detected during sorting.

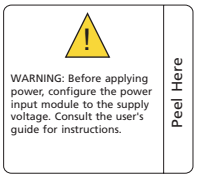
- Keep the sort collection chamber door closed when sorting into a plate. Do not place objects inside the sort collection chamber, and do not attempt to

access the plate until sorting is complete. The microtiter tray stage can move suddenly during the sorting process. Sorting continues even when the sort collection chamber door is open.

Precaution Labels

The following precaution labels appear on the BD FACSAria flow cytometer or fluidics cart to indicate a potential hazard. Do not remove these labels. Use appropriate precautions to avoid injury by the indicated hazard. See the previous sections for more information.

Label	Location(s)	Potential Hazard
	Waste tank, waste tank connectors on fluidics cart	Risk of exposure to biologically transmissible disease
	Waste tank cap	Risk of exposure to biologically transmissible disease
	Ethanol tank, ethanol tank connectors on fluidics cart	Highly flammable material
	Ethanol tank cap	Highly flammable material

Label	Location(s)	Potential Hazard
 CAUTION VISIBLE AND/OR INVISIBLE CLASS 3B LASER RADIATION WHEN OPEN. AVOID EXPOSURE TO THE BEAM. 335749 **CAUTION** VISIBLE AND/OR INVISIBLE CLASS 3B LASER RADIATION WHEN OPEN AND INTERLOCKS DEFEATED. AVOID EXPOSURE TO THE BEAM. 335751	On or near all removable covers and any place where the laser beam can emerge from the instrument	Risk of exposure to hazardous laser radiation
 Deflection Plates	Sort block door and deflection plates warning light	Dangerous voltage
	Behind sample injection chamber	Risk of crushing or pinching by moving parts
 CAUTION: MOVING PARTS	Floor of sort collection chamber	Risk of crushing or pinching by moving parts
 WARNING: Before applying power, configure the power input module to the supply voltage. Consult the user's guide for instructions. Peel Here	Power input on fluidics cart	Risk of instrument damage if wrong voltage applied[a]

a. **NOTE:** Voltage is set by your service engineer during installation.

Limitations

This instrument is for Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD Biosciences delivers software and workstations that are intended for running the instruments supplied by BD Biosciences. It is the responsibility of the buyer/user to ensure that all added electronic files including software and transport media are virus free. If the workstation is used for Internet access or purposes other than those specified by BD Biosciences, it is the buyer/user's responsibility to install and maintain up-to-date virus protection software. BD Biosciences does not make any warranty with respect to the workstation remaining virus free after installation. BD Biosciences is not liable for any claims related to or resulting from the buyer/user's failure to install and maintain virus protection.

1

BD FACSAria Instrument Components

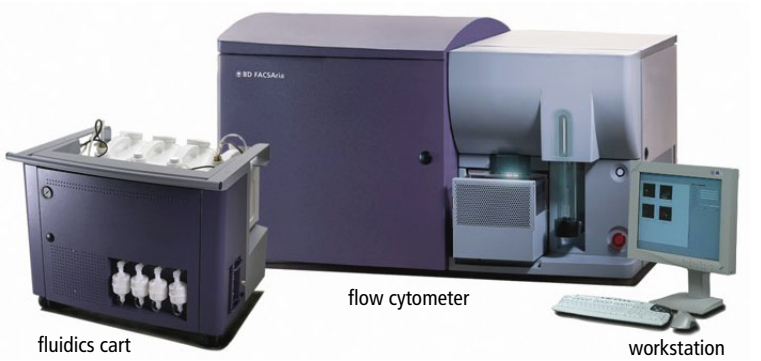
The BD FACSAria flow cytometer is the first fixed-alignment benchtop high-speed cell sorter. The instrument can be operated at varied pressures and can acquire up to 70,000 events per second. With its fixed-optics design and digital electronics, the BD FACSAria flow cytometer enables multicolor analysis of up to 13 fluorescent markers and two scatter parameters at a time.

The BD FACSAria system consists of three major components: a fluidics cart, a benchtop flow cytometer, and a workstation (see Figure 1-1 on page 22). Nearly all instrument functions are operated from within BD FACSDiva software.

For a description of the BD FACSAria system components, see the following sections. For technical information about how the instrument works, see Chapter 2.

- Fluidics Cart on page 22
- Flow Cytometer on page 26
- Workstation on page 44

Figure 1-1 BD FACSAria instrument components



Fluidics Cart

A separate fluidics cart supplies sheath and cleaning fluids and collects waste from the instrument (Figure 1-2). The self-contained fluidics cart supplies the required air pressure and vacuum, which eliminates the need for an external source (although the cart can be hooked up to an in-house air source, if one is available). The air pumps provide pressure from 2 to 75 psi to accommodate a variety of cell sorting applications. Air pressure is adjusted within BD FACSDiva software.

Figure 1-2 Fluidics cart

Containers and Connectors

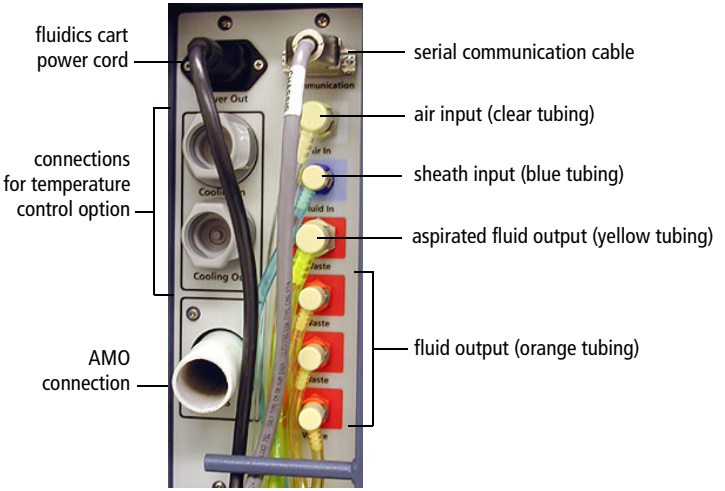
The fluidics cart holds four 10-L containers (two sheath and two waste), and three 5-L auxiliary cleaning fluid containers (Figure 1-3). The cart can also accommodate up to two BD FACSFlow™ 20-L cubitainers in place of the four 10-L containers.

Figure 1-3 Fluidics cart containers

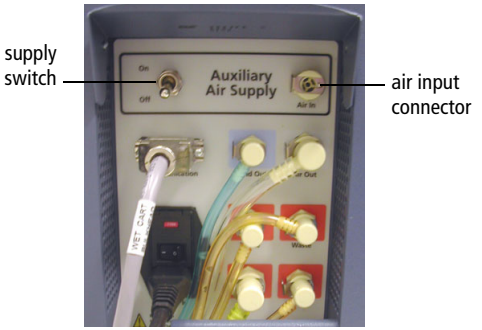
To prevent foaming, do not fill the containers with solutions containing a high concentration of detergent.

The fluidics cart connects directly to the flow cytometer unit via a power cord, fluid hoses, serial communication cable, and air line (Figure 1-4 on page 24). Receptacles for the aerosol management and temperature control options are also located within the connection panel. The position of the fluidics cart is constrained only by the length of the connecting cables and hoses, which extend up to 9 feet (2.4 m). Typically, the cart is placed to the left or underneath the cytometer.

Figure 1-4 Fluidics cart power and fluid line connectors



To connect the fluidics cart to an external air source, open the shutter above the Communication port, switch on the Auxiliary Air Supply, and attach the external air line to the air input connector. The external air supply should provide 80–100 psi.



If your laboratory runs the cytometer continuously and does not shut down at the end of the day, switch on the Auxiliary Air Supply for 15 seconds every 8 hours (every 4 hours in an extremely humid climate). Doing this empties the cart condensation trap and prevents excess moisture from overflowing the trap or causing cart damage.

Power and Operation

Power to the fluidics cart is supplied by the cytometer—the cart's fluid pumps are activated when the cytometer main power switch is turned on (see Power Panel on page 42).

⚠ Power to the fluidics cart is supplied and controlled through the flow cytometer. The fluidics cart voltage settings have been configured to match the supply voltage by your service engineer. To properly operate the fluidics cart, plug the fluidics cart power cord only into the power receptacle on the instrument (Figure 1-4 on page 24). Do not plug the power cord directly into a wall socket. Do not change the input voltage selection jumpers on the fluidics cart fuse holder.

⚠ When the stream is on, air pressure fluctuates between 80–100 psi (Figure 1-5). A pressure reading of less than 80 psi or greater than 100 psi indicates that the fluidics cart is not functioning properly. If this occurs, contact your BD Biosciences service representative for assistance. Do not operate the instrument outside the normal air pressure range.

Figure 1-5 Fluidics cart flow gauge

pressure gauge at approximately 88 psi

See the following sections for more information about the fluidics cart.

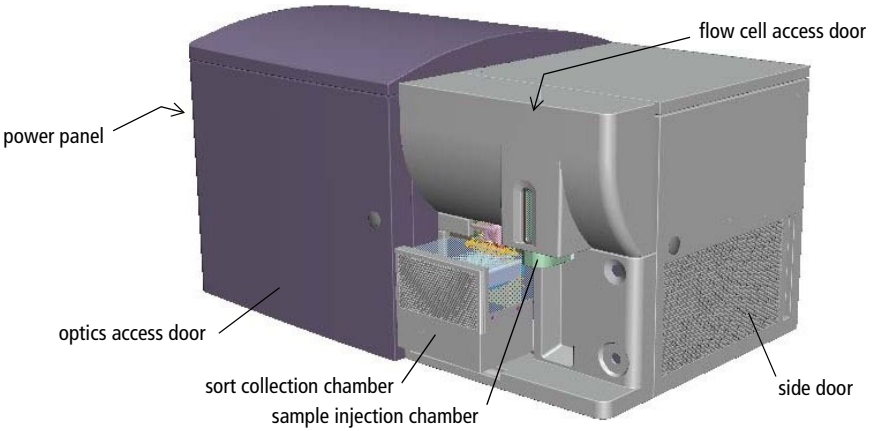
- Setting Up the Fluidics Cart on page 108
- Refilling Containers on page 109
- Emptying the Waste on page 110

- Scheduled Maintenance on page 175
- Fluidics Troubleshooting on page 229

Flow Cytometer

The benchtop flow cytometer contains the major components for all three subsystems (fluidics, optics, and electronics). The BD FACSAria instrument is relatively compact, with a much smaller footprint than most sorters with the same capabilities. The instrument can be set up on a typical laboratory benchtop or table, and it requires only a 20-amp electrical outlet. No special facilities are required.

Figure 1-6 BD FACSAria flow cytometer



To view the fluidics components, open the side door and lift the flow cell access door; to view the optics components, open the optics access door. The power panel and connectors are found on the left side of the instrument. Other electronic components are embedded within the instrument and do not need user adjustment.

The flow cell access door is equipped with a shutter mechanism that shuts off the laser light when the door is opened. To ensure there is no interruption to data acquisition, do not open the door while sorting or recording.

See the following sections for more information about the flow cytometer.

- Fluidics Components on this page
- Optics System on page 36
- Instrument Electronics on page 42

Fluidics Components

When the fluidics system is activated, sheath fluid is pumped from the fluidics cart into a plenum reservoir where the fluidics system is pressurized. From the reservoir, fluid is pumped into the cuvette flow cell where hydrodynamic focusing forces particles from the sample injection chamber through the cuvette in a single-file stream.

Within the cuvette flow cell, laser light is focused on the sample core stream. Fluorescent molecules excited by the different laser wavelengths are detected by the optics and analyzed by the electronics. Particles are then either transported to waste reservoirs via the waste aspirator, or sorted into a receptacle within the sort collection chamber.

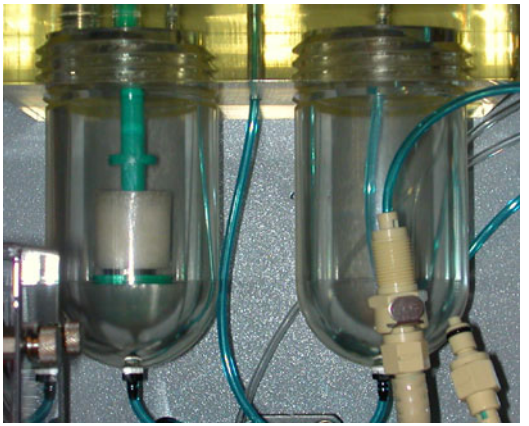
The following fluidics components are described in this section. For more information about fluidics, see Fluid Movement on page 46.

- Plenum Reservoirs on page 28
- Sample Injection Chamber on page 29
- Cuvette Flow Cell on page 30
- Nozzle on page 31
- Sort Block on page 32
- Sort Collection Chamber on page 35

Plenum Reservoirs

Sheath fluid is pumped from the fluidics cart into a plenum reservoir inside the side door of the BD FACSAria flow cytometer (Figure 1-7). From there, the fluid travels to a second reservoir where it is held and pressurized until it flows into the cuvette flow cell. This system eliminates pressure fluctuations by maintaining fluid levels and filtering out pump pulsation. As a result, the fluidics pressure does not vary with the level of fluid in the sheath containers.

Figure 1-7 Plenum reservoirs



Note that the plenum reservoirs can be unscrewed and removed for cleaning, if needed. See Cleaning the Plenum Reservoirs on page 184.

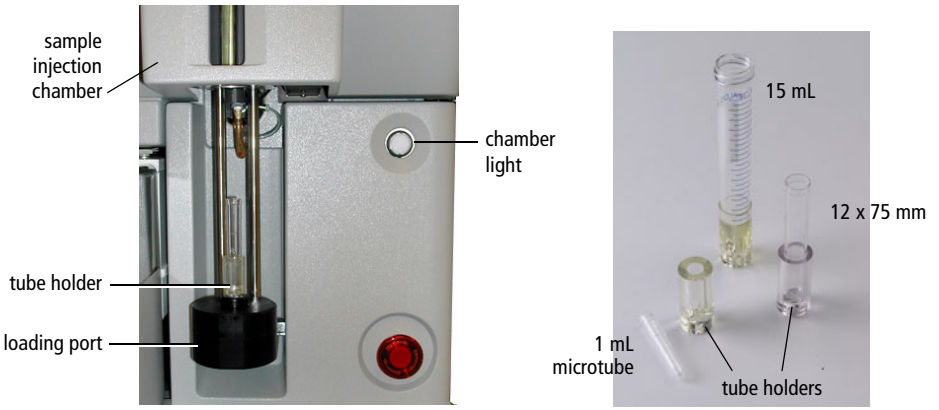
Sample Injection Chamber

The sample injection chamber is where sample is introduced into the flow cytometer. During acquisition, the chamber is pressurized to force sample toward the cuvette flow cell.

Samples can be agitated and temperature-controlled within the sample injection chamber using controls in the software (see Fluidics Controls on page 81). You can view the amount of fluid remaining in your sample tube by pressing the chamber light button shown in Figure 1-8.

⚠ Do not use the chamber light for long periods with samples stained with light-sensitive reagents.

Figure 1-8 Sample injection chamber and tube holders



A variety of tube holders are provided with the instrument to accommodate tubes from 15-mL centrifuge tubes to 1.0-mL microtubes (Figure 1-8). (For a list of compatible tubes, see Labware on page 240.) To load a tube, install the appropriate-size tube holder in the loading port, and place a tube in the holder. When the Load button is clicked in the software (see Acquisition Controls on page 89), the loading port rises to enclose the tube within the chamber.

After a tube is loaded, the Load button changes to Unload. Use the Unload button to lower the loading port after data has been recorded. After each tube is unloaded, sheath fluid flushes the sample tubing inside and out to reduce

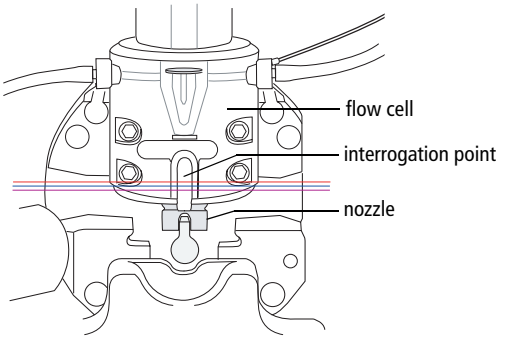
potential sample carryover. Using a special cleaning mode, the entire sample injection chamber can be decontaminated with ethanol. See Clean Bulk Injection Chamber on page 185 for more information.

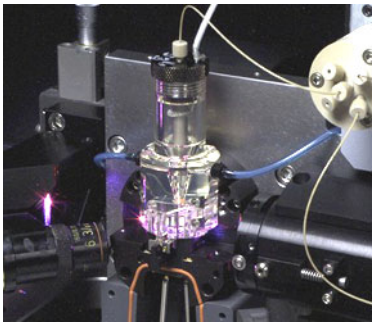
To prevent injury from moving parts, keep your hands and clothing away from the loading port when a tube is loading or unloading. Do not place objects under the loading port.

Cuvette Flow Cell

The cuvette flow cell is the heart of the BD FACSAria instrument (Figure 1-9). Within the flow cell, hydrodynamic focusing forces particles through the cuvette in a single-file stream, where laser light intercepts the stream at the sample interrogation point.

Figure 1-9 Cuvette flow cell





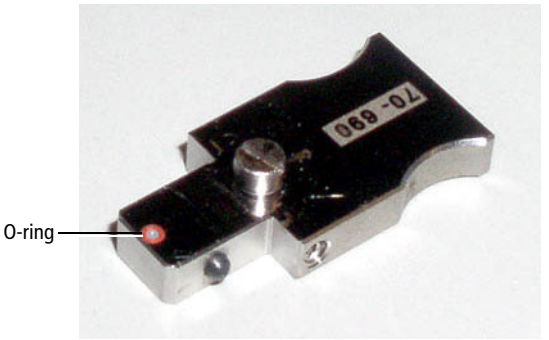
laser light intercepting flow cell

The unique flow cell design permits particles to flow through the cuvette at a low velocity (approximately 13 cm/sec for the high Sort Setup), allowing longer exposure to laser energy. The cuvette is gel-coupled to the fluorescent objective lens to transmit the greatest amount of emitted light from the interrogation point to the collection optics (see Optics System on page 36). After passing through the cuvette, the stream is accelerated (to approximately 69 cm/sec with the 70-µm nozzle) as it enters the nozzle tip, where the drop drive breaks the stream into droplets for sorting.

Nozzle

The BD FACSAria instrument is provided with two adjustable nozzles, 70 and 100 µm, that accommodate a variety of particle sizes. The nozzle is keyed to a fixed position at the end of the cuvette. Because the nozzle is below the interrogation point, optical alignment is not affected when the nozzle is changed.

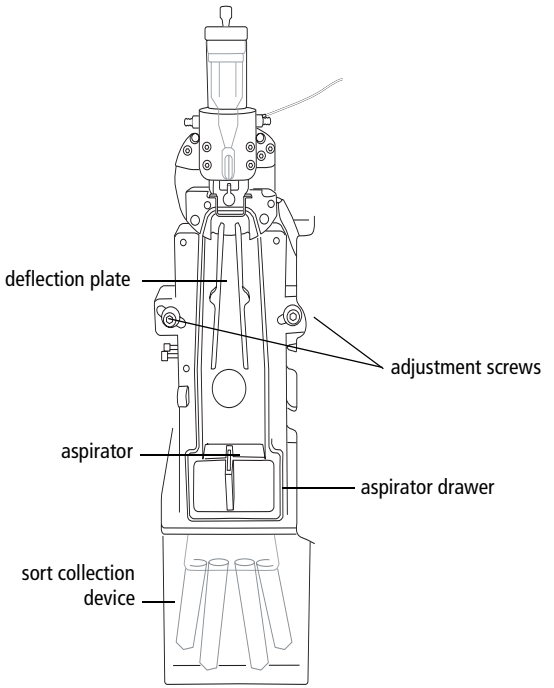
Figure 1-10 Nozzle



Sort Block

After leaving the nozzle, particles pass through the sort block where they are either transported to waste via the waste aspirator, or sorted into a collection device in the sort collection chamber. The sort block houses the high-voltage deflection plates, along with the aspirator and aspirator drawer (Figure 1-11).

Figure 1-11 Sort block with door open



Note that the entire sort block assembly can be rotated on a fixed pivot point to adjust the position of the stream in the waste aspirator. If the keyed stream position differs between an old nozzle and a new one, the stream might not hit the center of the aspirator after the nozzle is changed. In this case, you can change the angle of the sort block by loosening the adjustment screws on either side of the deflection plates and rotating the sort block. Tighten the screws when the stream is re-centered in the aspirator.

Deflection Plates

The high-voltage deflection plates are used to deflect side streams during sorting. The plates are turned on and off using the Voltage control in the Side Stream window (see Side Stream Formation on page 67). A red warning light is illuminated whenever the plate voltage is on (Figure 1-13 on page 34).

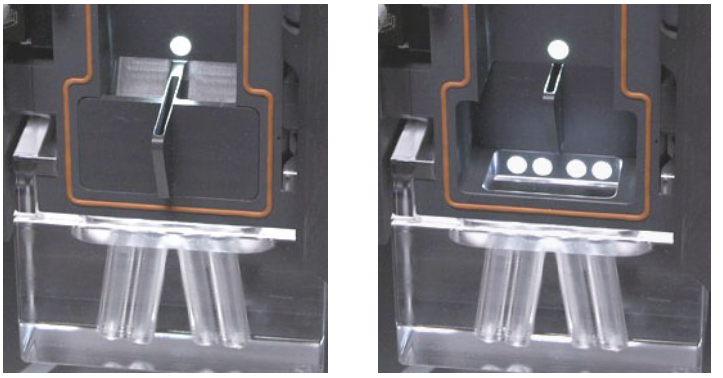
⚠ A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the voltage warning light is illuminated, or when the software indicates that the plate voltage is on. The plates remain energized even when the sort block door is opened.

Aspirator Drawer

The aspirator drawer keeps the sort collection tubes covered until sorting begins (Figure 1-12). You can open and close the drawer using a control in the Sort Layout or Side Stream window (see Using Sorting Controls on page 98). When the Sweet Spot is on and a clog is detected during sorting, the drawer closes automatically to protect your sort collection tubes.

⚠ To avoid pinching your hands or fingers in the drawer, keep your hands away from the sort block during sorting.

Figure 1-12 Aspirator drawer closed (left) vs open (right)

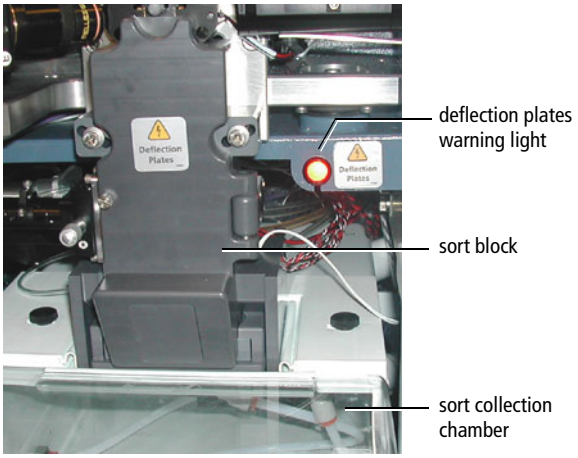


Aerosol Management

During sample acquisition and sorting, the sort block door should be kept closed to help contain potential aerosols (Figure 1-13).

Cell sorters that use droplet generation methods, like the BD FACSAria, can produce aerosols around the sample stream. Inhalation or contact with aerosols exposes you to biologically transmissible diseases. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door closed during sorting. If you need to access the sort block, turn off the stream before opening the door.

Figure 1-13 Sort block with door closed



Additional aerosol removal is provided by the BD™ Aerosol Management Option (AMO). The AMO is an optional device that evacuates the sort collection chamber during sorting. The AMO is equipped with a 0.01-µm pore, ultra-low penetrating air (ULPA) filter to trap aerosolized particles. The AMO pump evacuates the sort collection chamber 16 times per minute when operating at 20% capacity in normal evacuation mode, and 69 times per minute at 100% capacity in rapid evacuation mode.

Sort Collection Chamber

Collection devices are installed in the sort collection chamber to collect sorted samples during sorting. The following collection devices are provided with the instrument to accommodate a variety of tube sizes:

- Four-way 1-mL collection tube holder
- Four-way 12 x 75-mm collection tube holder
- Two-way 12 x 75-mm collection tube holder
- Two-way 15-mL collection tube holder

An automated cell deposition unit (ACDU) that sorts into multiwell plates and onto microscope slides is available as an option. BD Biosciences also offers a temperature-control option to maintain the temperature of sorted samples during sorting.

Figure 1-14 Sort collection devices

collection devices

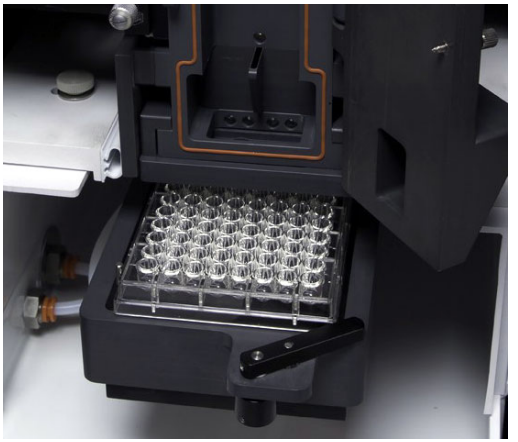


plate loader

The sort collection chamber door should be kept closed when sorting into a plate. The door keeps the chamber free of dust and other air-borne particles, and seals the chamber during aerosol evacuation for instruments equipped with the AMO.

Optics System

The BD FACSAria cytometer uses innovative designs for both the excitation optics and collection optics. The optics can be viewed by opening the optics access door and the flow cell access door. See the following sections for more information.

⚠ The flow cell access door is equipped with a shutter mechanism that shuts off the laser light when the door is opened. To ensure there is no interruption to data acquisition, do not open the door while sorting or recording.

Excitation Optics

The excitation optics consist of lasers, fiber optic cables, beam-shaping prisms, and an achromatic focusing lens, as shown in Figure 1-15 on page 37. For information about how signals are generated, see Signal Generation on page 50.

The BD FACSAria instrument uses low-powered, air-cooled and solid-state lasers that do not have special power and cooling requirements. The instrument comes standard with 488-nm and 633-nm lasers. A third 407-nm laser is available as an option.

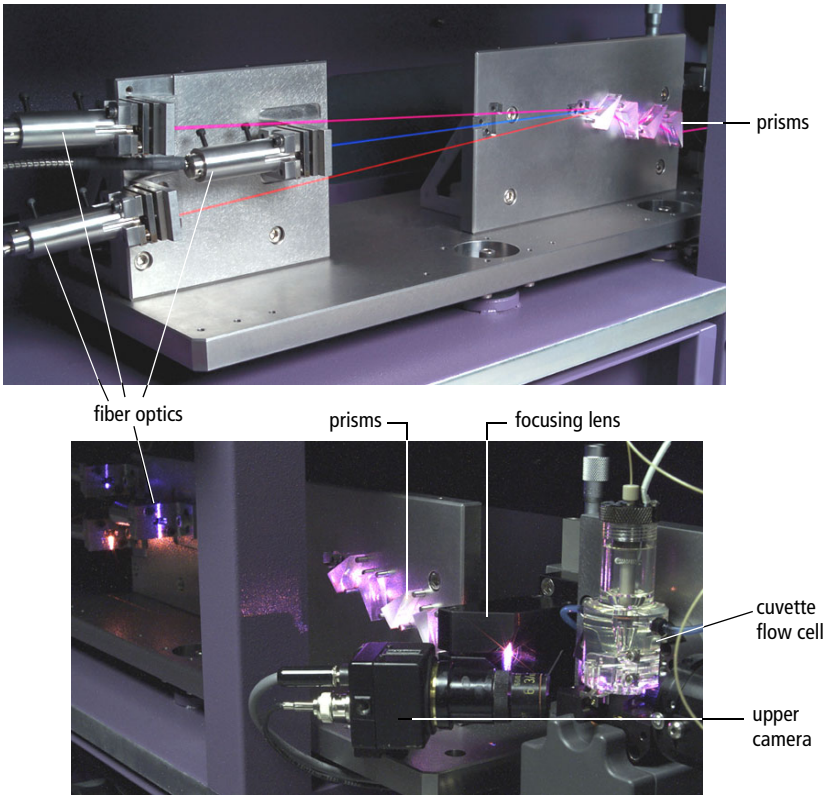
Laser	Wavelength (nm)	Min. Power[a] (mW)	Commonly Used Fluorochromes
Coherent® Sapphire™ Solid State	488 (blue)	13	FITC, PE[b], PE-Texas Red®, PerCP, PerCP-Cy5.5, PE-Cy7, PI
JDS Uniphase™ HeNe Air Cooled	633 (red)	11	APC, APC-Cy7
Point Source Violet Solid State (optional)	407 (violet)	10	Alexa Fluor®, Cascade Blue®, Pacific Blue™, DAPI, Hoechst

a. Measured out of fiber optic cable
b. Patents: PE and APC—US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942; PerCP—US 4,876,190; Cy—US 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027; PE-Cy7—US 4,542,104; APC-Cy7—US 5,714,386

Fiber optics direct the laser light in a precise and constant manner onto beam-shaping prisms, which in turn transmit the laser light to a focusing lens. The lens focuses the laser light onto the sample core stream within the cuvette flow cell (Figure 1-15). The lasers are positioned on the sample stream for optimal generation of signals. Since the optical pathway and sample core stream are fixed, optimization is constant from day to day.

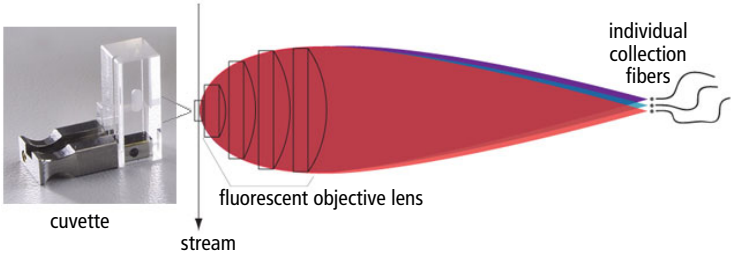
Lasers emit intense, coherent electromagnetic radiation that can cause irreparable damage to human skin and eyes. To prevent retinal burns and possible blindness, do not remove laser shielding, adjust laser controls, or attempt to service the instrument any place where laser warning labels are attached. See Precaution Labels on page xviii for the placement of laser warning labels.

Figure 1-15 Excitation optics pathway (behind laser shielding)

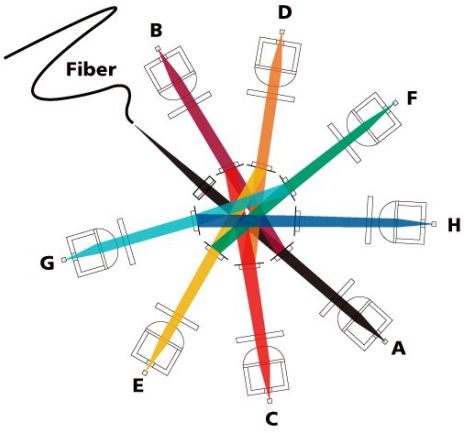


Collection Optics

From the cuvette flow cell, laser light is collected by a fluorescent objective lens that is gel-coupled to the cuvette to transmit the maximum amount of light. The lens collects and focuses fluorescent light emitted at each of the laser focal points onto individual collection fibers. These fibers transfer the emitted light to the collection optics, as shown in the following figure.



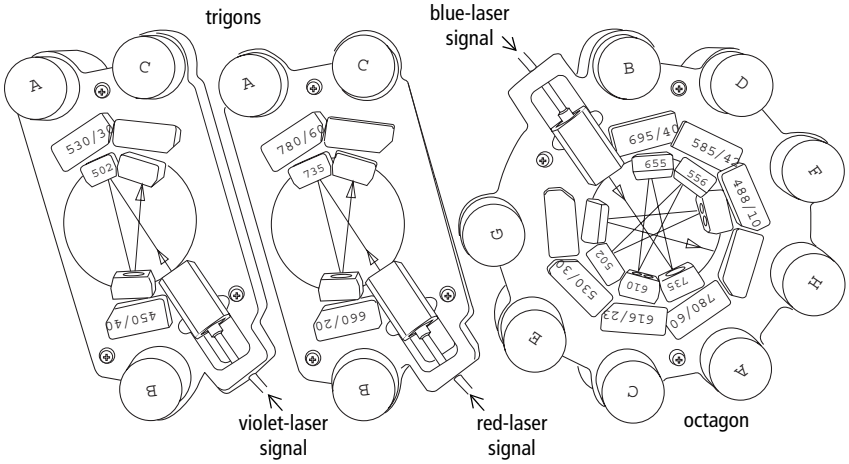
The collection optics are set up in octagon- and trigon-shaped arrays that are engineered to maximize signal detection from each laser. This is accomplished by transmitting the highest wavelengths to the first photomultiplier tube (PMT), and reflecting lower wavelengths to the next PMT through a series of long-pass dichroic mirrors. Bandpass filters in front of each PMT allow fine-tuning of the spectral wavelengths that need to be collected. Since reflection is more efficient than transmittance, this design greatly increases the multicolor detection capabilities of the instrument.



Detectors

A standard system is equipped with an octagon containing six PMTs, which detect light from the 488-nm (blue) laser, and a trigon containing two PMTs, which detect light from the 633-nm (red) laser. The third-laser option adds an additional trigon containing two PMTs to detect light from the 407-nm (violet) laser. Optical upgrades are available to fill the remaining PMT slots for a two- or three-laser system. A fully-equipped system can detect up to 13 fluorescent parameters and two scatter parameters at a time.

Figure 1-16 Fully loaded detector arrays



At installation, the octagon and trigon arrays are set up with the filter and mirror combinations shown in Table 1-1 on page 40. You can rearrange these configurations according to the type of fluorochromes in your experiment. See Application Options on page 242 for more information.

Table 1-1 Default setup for detector arrays

Detector Array (Laser)	PMT	LP Mirror	BP Filter	Intended Dye
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	655	695/40 675/20	PerCP-Cy5.5 or PI PerCP
	C	610	616/23	PE-Texas Red
	D	556	585/42	PE or PI
	E	502	530/30	FITC
	F	—	488/10[a]	Side scatter (SSC)
Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	—	660/20	APC
Trigon (407-nm violet laser)	A	502	530/30	Alexa Fluor 430
	B	—	450/40	Cascade blue, Pacific blue, DAPI, Hoechst, Alexa Fluor 405

a. The optical holder for the 488/10 BP filter also includes a 1.0 ND filter.

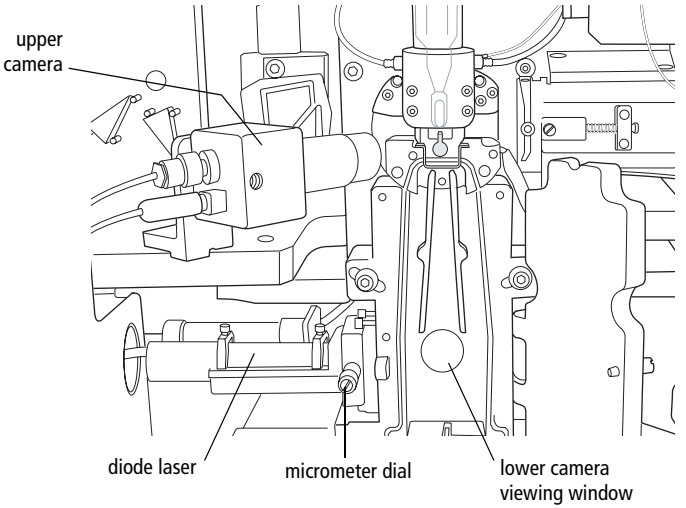
Stream-Viewing Optics

The BD FACSAria instrument is equipped with additional optical components that are used to view the stream (Figure 1-17).

- The upper camera generates an image used to monitor drop formation. It is focused on the stream, just below the nozzle, to provide an image of the drop breakoff.

- The lower camera generates an image used for the BD FACS™ Accudrop* function. It enhances the ability to see side streams and assists in setting an accurate drop delay value.

Figure 1-17 Stream-viewing optics



Special image-processing software allows you to view the stream images from each camera within separate windows in BD FACSDiva software. See Sorting on page 62 for more information about viewing the streams and to learn how Accudrop components are used to determine the drop delay.

* US Patent No. 6,372,506

Instrument Electronics

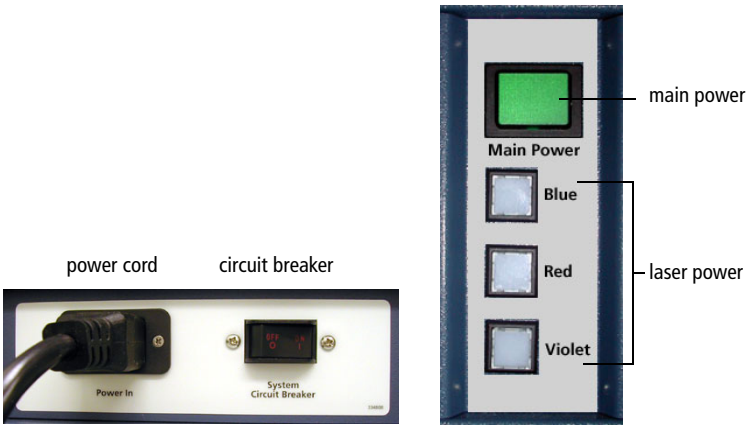
The electronic components consist of power controls and connectors along with processing boards in the card cage. This section describes only user-adjustable instrument electronics; for more information about the electronics, see Signal Detection on page 52.

Power Panel

Power to the instrument, lasers, and fluidics cart is supplied by a power cord plugged directly into a standard electrical outlet; there are no special facilities requirements. The main power button turns on the instrument and fluidics cart, and enables the laser power buttons. To turn on a laser, press the corresponding button. The button lights up when the power is on.

⚠ Do not turn on the lasers until you have activated the stream. Running the lasers without activating the stream can degrade the performance of the cuvette flow cell.

Figure 1-18 Flow cytometer power panel



The instrument circuit breaker is located next to the power cord (Figure 1-18). The switch will need to be reset if there is a power surge in the laboratory.

Emergency Stop Button

The emergency stop button to the right of the loading port (Figure 1-19) is a safety feature that can be used to halt the movement of the loading port and ACDU stage in case of need. The button lights up when a tube is loading to remind you to keep your hands away from the loading port.

Figure 1-19 Emergency stop button

The following occur when this button is pushed:

- The tube is unloaded from the sample injection chamber.
- The ACDU stage (if in use) stops moving.
- The stream is turned off.
- The deflection plate voltage is turned off.
- The aspirator drawer (if open) closes to protect sort collection tubes.
- A warning message appears on the screen.

Do not reset the button until the message appears. To do so, turn the button clockwise until the light turns off and the button returns to its original position.

NOTICE The emergency stop button does not turn off the lasers or shut down the instrument main power.

Workstation

Data acquisition and analysis, as well as most BD FACSAria instrument functions, are controlled by BD FACSDiva software on a PC workstation. The workstation includes a desktop computer, one or two monitors, and a color printer, and is equipped with the following applications:

- Microsoft Windows operating system
- BD FACSDiva software
  - Data acquisition and analysis
  - Automatic fluidics startup, shutdown, and cleaning modes
  - Sort setup, stream monitoring, clog detection, and recovery
- Supporting documentation for the software

For information about BD FACSAria–specific software features, see Chapter 2, Theory of Operation, and Chapter 3, Using BD FACSDiva Software. For general software information, refer to the *BD FACSDiva Software Reference Manual*.

☑ **Tip** For easy access to the *BD FACSDiva Software Reference Manual*, choose Software Manual from the BD FACSDiva Help menu.

2

Theory of Operation

This chapter describes how the BD FACSAria instrument works and how BD FACSDiva software components are used to operate different system components. For a general overview of the software, see Chapter 3.

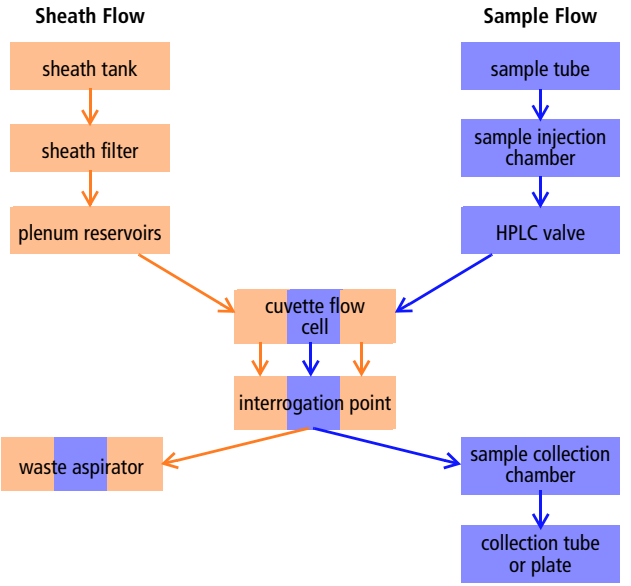
See the following sections for a description of these BD FACSAria functions:

- Fluid Movement on page 46
- Signal Generation on page 50
- Signal Detection on page 52
- Electronic Processing on page 59
- Sorting on page 62

Fluid Movement

The fluidics system is responsible for moving particles from the sample injection chamber through the cuvette flow cell for interrogation, and then to waste or into a collection device during sorting (Figure 2-1). The following sections describe the controls used to move fluids through the BD FACSAria fluidics system.

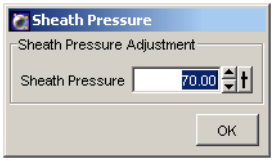
Figure 2-1 Fluidic movement via the fluidics system



Sheath Flow

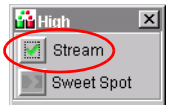
When you turn on the instrument, the fluidic pumps start up. The fluidics system is activated when you choose the Fluidics Startup command in BD FACSDiva software. During fluidics startup, sheath fluid is pumped from the sheath container through a 0.22-µm filter into the plenum reservoirs. The reservoirs are pressurized to deliver sheath fluid to the cuvette flow cell at a constant pressure. You can view the current sheath pressure using the Instrument > Sheath Pressure command (Figure 2-2 on page 47).

Figure 2-2 Sheath pressure level



After fluidics startup, sheath flow is controlled using the Stream button in the Breakoff window, which is labelled with the chosen sort pressure (Figure 2-3). When clicked, the button changes from a red "X" to a green checkmark, and sheath flows through the cuvette flow cell at the rate that is specified in the Sheath Pressure window. (See Drop Formation on page 63 for a complete description of the Breakoff window.)

Figure 2-3 Stream control in Breakoff window



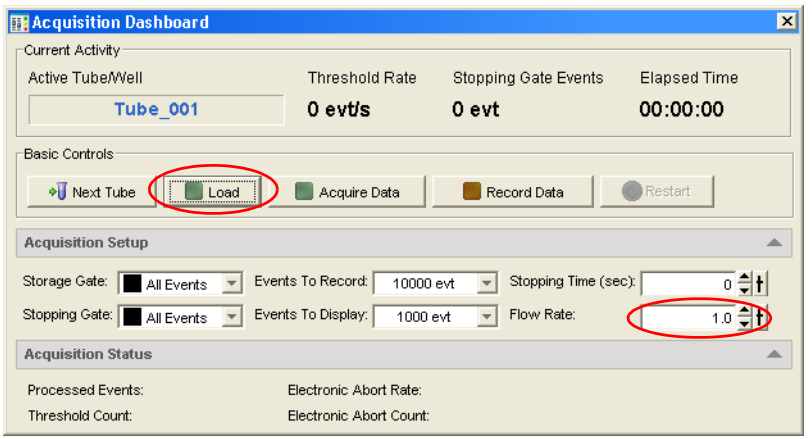
As a general rule, the sheath pressure level is set by choosing a Sort Setup option from the Sort menu, rather than by adjusting the Sheath Pressure control. Each Sort Setup option is optimized at a preset sheath pressure. If you change the sheath pressure, a multitude of other values will be affected, including the drop drive frequency, drop charge levels, laser delay, area scaling factor, and other values. For more information, see Sort Setup on page 92.

Sample Flow

Sample is introduced into the cuvette when the Load button is clicked in the Acquisition Dashboard (Figure 2-4). After Load is clicked, the loading port rises to enclose the tube within the sample injection chamber. The chamber is automatically pressurized and the chamber pressure forces sample through the sample line into the cuvette flow cell. To stop sample flow after a tube is loaded, click the Stop Acquiring button.

The sample flow rate is specified using the Flow Rate field in the Acquisition Dashboard. You can adjust the Flow Rate from 1–11, which corresponds to approximately 10–120 µL/min.

Figure 2-4 Load button and Flow Rate field in Acquisition Dashboard

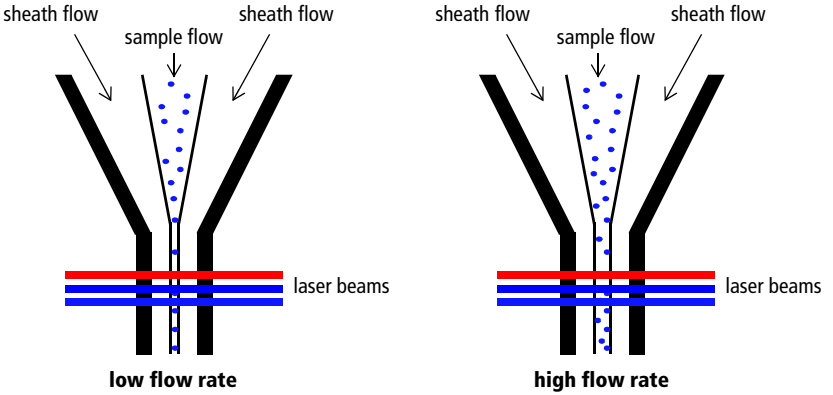


Note that the relatively longer sample tubing on the BD FACSAria instrument results in a different flow rate between cells and beads. Thus, absolute counting using BD Trucount™ beads can give erroneous results.

Hydrodynamic Focusing

In the flow cell, pressurized sheath fluid surrounds the sample fluid to hydrodynamically focus the core stream of suspended cells into the center of the cuvette, where the particles are intercepted by the laser beam.

The difference in pressure between the sheath fluid and the sample fluid can be used to vary the diameter of the sample core. A lower difference results in a relatively narrow core stream, while a higher difference results in a wider sample stream.



Ideally, you want the core stream at its minimum diameter so that cells pass through the laser beam in a single-file stream. However, depending on your application, a lower resolution might be acceptable in order to acquire the data more quickly. For example, a higher flow rate is generally used for qualitative measurements such as immunophenotyping—the data is less resolved but is acquired more quickly. A lower flow rate is generally used in applications where greater resolution is critical.

For sorting applications, sorting results are optimized at lower flow rates (<8.0).

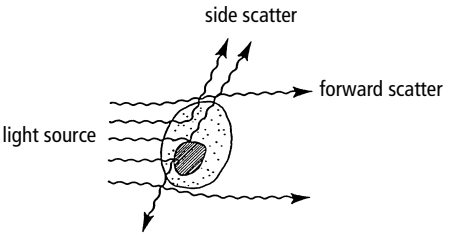
Signal Generation

The following sections describe how signals are generated when cells or particles intercept the laser within the cuvette flow cell.

Light Scatter

When a cell or particle passes through a focused laser beam, laser light is scattered in all directions. Light that scatters axial to the laser beam is called forward scatter (FSC); light that scatters perpendicular to the laser beam is called side scatter (SSC). FSC and SSC are related to certain physical properties of cells:

- FSC—indicates relative differences in the size of the cells or particles
- SSC—indicates relative differences in the internal complexity or granularity of the cells or particles



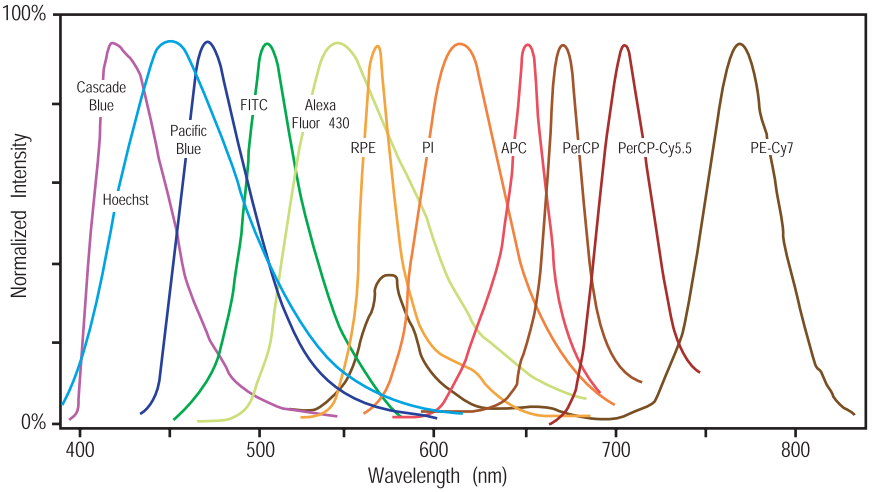
Fluorescent Signal

When cells or particles stained with fluorochrome-conjugated antibodies or other dyes pass through a laser beam, the dyes can absorb photons (energy) and be promoted to an excited electronic state. In returning to their ground state, the dyes release energy, most of which is emitted as light. This light emission is known as fluorescence.

Fluorescence is always a longer wavelength (lower-energy photon) than the excitation wavelength. The difference between the excitation wavelength and the emission wavelength is known as the Stokes shift. Some fluorescent compounds such as PerCP exhibit a large Stokes shift, absorbing blue light (488 nm) and emitting red light (675 nm), while other fluorochromes such as FITC have a smaller Stokes shift, absorbing blue light and emitting green light (530 nm).

The emission spectra for some commonly used fluorochromes are shown in Figure 2-5. See Table 1-1 on page 40 for the corresponding detectors.

Figure 2-5 Emission spectra of commonly used fluorochromes



Signal Detection

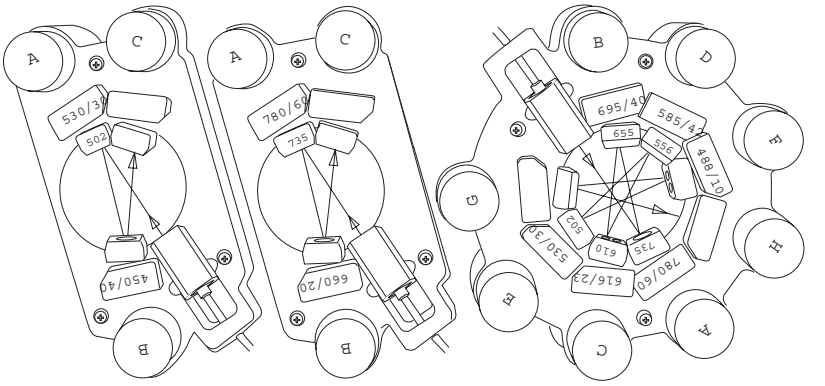
From the cuvette flow cell, scattered and fluorescent light is collected by the fluorescent objective lens. The lens collects and focuses fluorescent light emitted at each of the laser focal points onto individual collection fibers. These fibers transfer the emitted light to the individual detector arrays.

The following sections describes how laser light is detected and translated into signals that can be displayed in a plot.

Detector Arrays

There are two types of detector arrays in the BD FACSAria flow cytometer: an octagon and trigons. The octagon detects SSC and up to seven fluorescent signals excited by the 488-nm (blue) laser. The trigons detect fluorescent signals excited by the 633-nm (red) and 407-nm (violet) lasers, respectively. Each trigon can detect up to three fluorescent channels. (An optical upgrade is required to fill all detection channels.)

Figure 2-6 Octagon and trigon detector arrays



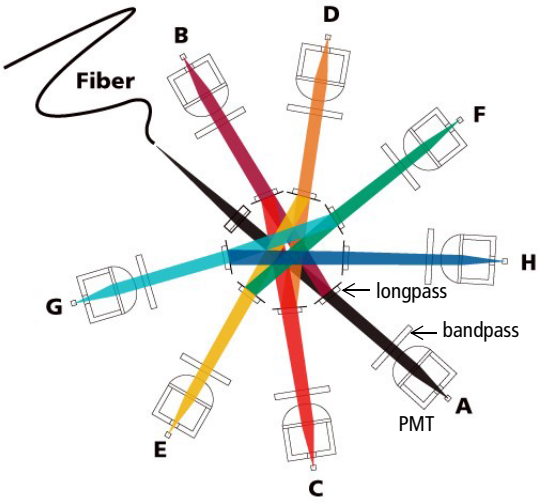
violet-laser signal red-laser signal blue-laser signal

Each detector array houses dichroic and bandpass filters, which steer and filter the emitted light, and photomultiplier tubes (PMTs), which detect light signals.

Filters

Optical filters modify the spectral distribution of light scatter and fluorescence directed to the detectors. Three kinds of filters are used in the detector arrays: longpass (LP) filters are used to steer light between the detectors within a detector array, while bandpass (BP) and neutral density (ND) filters allow fine-tuning of the spectral wavelengths that need to be collected (Figure 2-7).

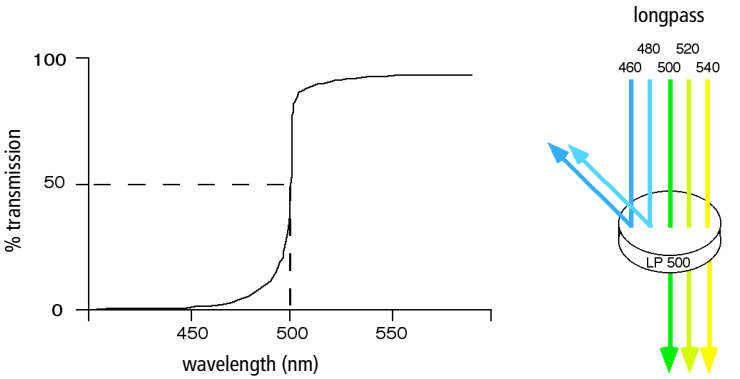
Figure 2-7 Detectors and filters in octagon array



Longpass Filters

Longpass (LP) filters pass wavelengths longer than the filter rating and reflect shorter wavelengths. For example, a 500 LP filter permits wavelengths longer than 500 nm to pass through it and reflects wavelengths shorter than 500 nm (Figure 2-8).

Figure 2-8 Light reflection by longpass filter



Dichroic filters that are used to direct different color light signals to different detectors are called dichroic mirrors or beam splitters.

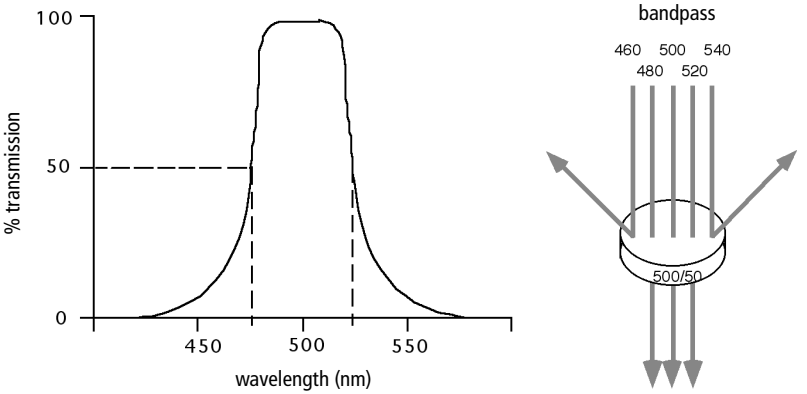
Although dichroic mirrors have the properties of LP optical filters, you cannot necessarily use any type of LP filter as a beam splitter. A beam splitter must have a surface coating that reflects certain wavelengths, but many types of LP filters are absorbance filters that do not have any specific reflective characteristics. Also, optical filters and beam splitters are rated at a specific angle of incidence. When used as a beam splitter, they are placed at an angle relative to the light source. Their optical properties are therefore designed for that angle of incidence.

The detector arrays use LP dichroic mirrors to steer progressively shorter wavelengths of light to the next PMT in the array. For example, in the octagon array, light first passes through a 735 LP filter in the A position, followed by a 655 LP filter in the B position. Thus, wavelengths longer than 735 nm are detected at PMT-A; all wavelengths <735 nm are reflected to PMT-B. Wavelengths between 655 nm and 735 nm are detected at PMT-B; all wavelengths <655 nm are reflected to PMT-C, and so on. For a list of the longpass filters used in the detector arrays, see Table 1-1 on page 40.

Bandpass Filters

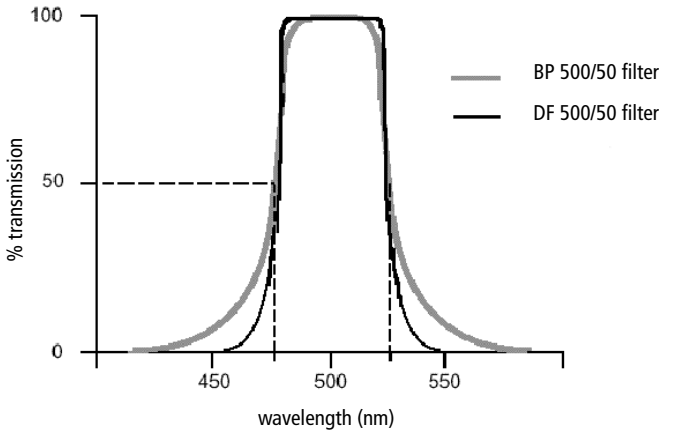
Bandpass (BP) filters transmit a relatively narrow range or band of light. Bandpass filters are typically designated by two numbers. The first number indicates the center wavelength and the second refers to the width of the band of light that is passed. For example, a 500/50 BP filter transmits light that is centered at 500 nm and has a total bandwidth of 50 nm. Therefore, this filter transmits light between 475 and 525 nm (Figure 2-9).

Figure 2-9 Light transmittance by bandpass filters



Discriminating (DF) filters have the same general function—they transmit a relatively narrow band of light. The principal difference between BP and DF filters is their construction. DF filters have more cavities or layers of optical coatings, resulting in a steeper transmission curve than the curve for a BP filter. This steep slope means that a DF filter is better at blocking light outside the rated bandwidth of the filter. See Figure 2-10 on page 56.

Figure 2-10 Bandpass (BP) vs discriminating (DF) filters

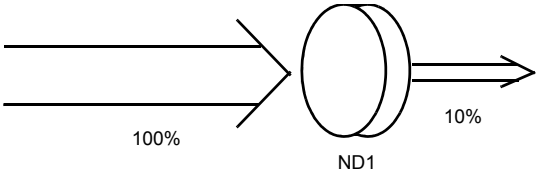


In the detector arrays, DF filters block high-intensity laser light and filter the remaining light to ensure that only the required wavelengths reach their intended detector. For example, in the octagon array, PMT-A has a 780/60 DF filter in front of it, which transmits light of 750–810 nm. Thus, the only wavelengths that will reach the A detector are those between 750 and 810 nm.

For optimal detection of fluorescent light, a bandpass filter must always be installed in front of each detector. For a list of the bandpass filters used in the detector arrays, see Table 1-1 on page 40.

Neutral Density Filters

Neutral density (ND) filters transmit a fixed percentage of light, reducing the transmitted intensity of all wavelengths equally. ND filters are neutral with respect to wavelength.



The ND filters on the BD FACSAria allow approximately 10% of the light to be transmitted. You can find them in front of the FSC and SSC detectors.

For applications involving small particles (eg, bacteria or platelets), you might need to remove the FSC ND filter. See Removing/Installing the FSC ND Filter on page 248. For applications involving large particles where events appear off scale on the FSC axis with a gain of zero, the FSC ND filter is needed to decrease FSC signal and keep the events on scale.

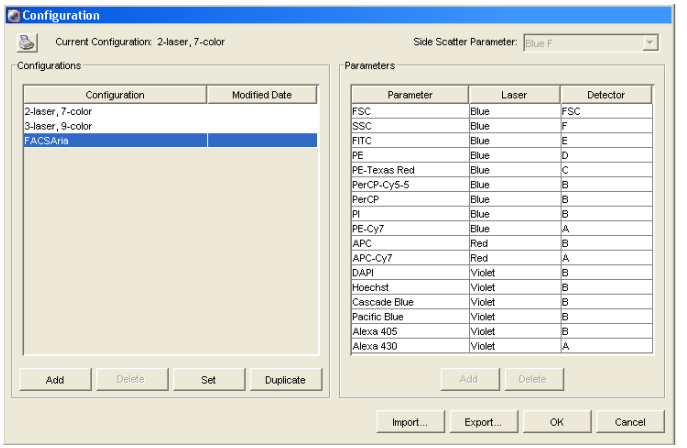
Detectors

Detectors within each detector array convert light signals into electrical signals that can be processed by the electronics system.

There are two types of signal detectors in the BD FACSAria flow cytometer: the photodiode and photomultiplier tubes (PMTs). The photodiode is less sensitive to light signals than the PMTs, thus is used to detect the stronger FSC signal. The photodiode detects FSC light from the blue laser, and is stored outside the detector arrays. PMTs are used to detect the weaker signals generated by SSC and all fluorescence channels. The octagon can hold up to eight PMTs; each trigon can contain up to three PMTs.

Each PMT detects only one fluorochrome at a time. In BD FACSDiva software, the Instrument Configuration dialog box lets you define which fluorochromes or cell parameters will be measured at each PMT detector. If more than one fluorochrome is measured using the same PMT, you can add additional parameters to your configuration and choose the appropriate fluorochrome within your software experiment. See Instrument Configuration on page 85 for more information.

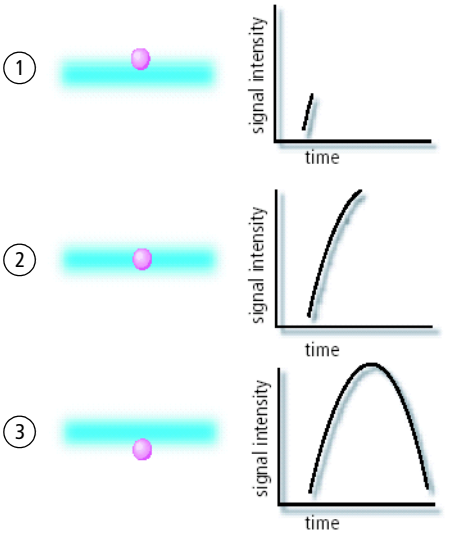
Figure 2-11 Default BD FACSAria instrument configurations



Electronic Processing

As cells or other particles pass through the focused laser beams, they scatter the laser light and can emit fluorescence. Because each laser beam is focused on a small spot and particles move rapidly through the flow cell, the scatter or fluorescence emission has a very brief duration—only a few microseconds. The PMTs convert this brief flash of light into an electrical signal called a pulse (Figure 2-12).

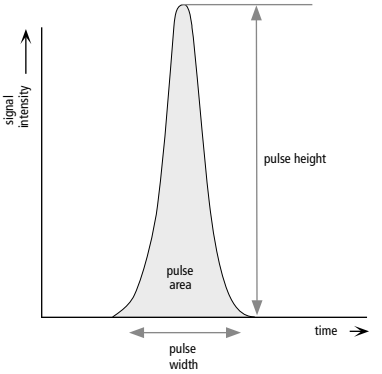
Figure 2-12 Anatomy of a pulse



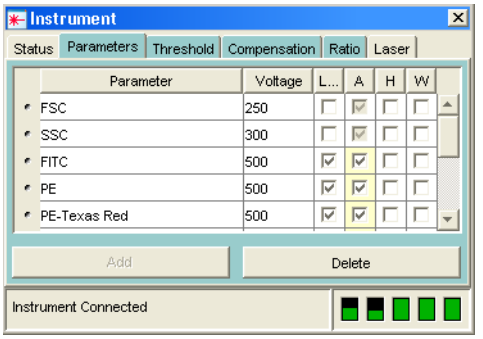
1	A pulse begins when a particle enters the laser beam. At this point, both the beam intensity and signal intensity are low.
2	The pulse reaches a maximum intensity or height when the particle reaches the middle of the beam, where the beam and signal intensity are the brightest. The peak intensity, or height of the pulse, is measured at this point.
3	As the particle leaves the beam, the pulse trails off.

Pulse Parameters

A *parameter* is a pulse property that is generated by a single photomultiplier tube or photodiode, measuring fluorescent or scattered light. You can measure three characteristics of a pulse: the area, height, or width. The pulse height measures the maximum digitized value for the pulse; the pulse area calculates the sum of all height areas for the pulse; and the pulse width calculates $\frac{\text{area}}{\text{height}} \times 64000$.



You can select which pulse characteristics you want to measure for each parameter using the Parameters tab. Pulse area (A) is measured by default. The Parameters tab also contains Voltage controls that allow you to amplify signals by applying a voltage to PMTs or an electronic gain to FSC signal. As the voltage is increased, the detector sensitivity increases, resulting in increased signal. As the voltage is decreased, the detector sensitivity decreases, resulting in decreased signal.



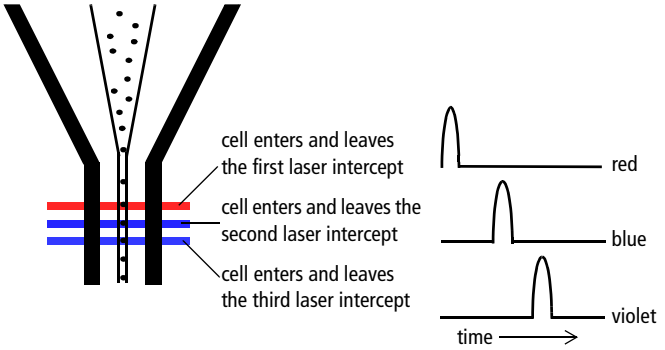
Digital data is displayed on an 18-bit linear scale, from 2.6–262,144. Select the Log checkbox to convert the display to a log scale. The Experiment Inspector contains an option to display log data on a four- or five-decade scale. (Refer to the *BD FACSDiva Software Reference Manual* for more information.)

Laser Delay

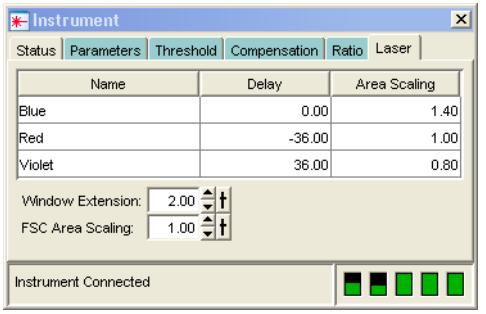
Sample interrogation takes place within the cuvette flow cell. As described in Optics System on page 36, fiber optic cables are used to direct laser light through a series of prisms that focus each laser on the stream at a separate position. This allows optimal detection of fluorescent signal from each laser with minimal cross-contamination from the other beams.

In a three-laser system, the red laser intercepts the stream first, followed by the blue and then the violet laser. Because the laser signals are spatially separated, there is a slight delay between the detection of each laser's signal (Figure 2-13).

Figure 2-13 Signal separation over time



The Delay factor in BD FACSDiva software is used to realign the signals so they can be measured and displayed on the same time scale. Note that signals are aligned with respect to the blue laser, so the red laser signals always have a negative Delay value.



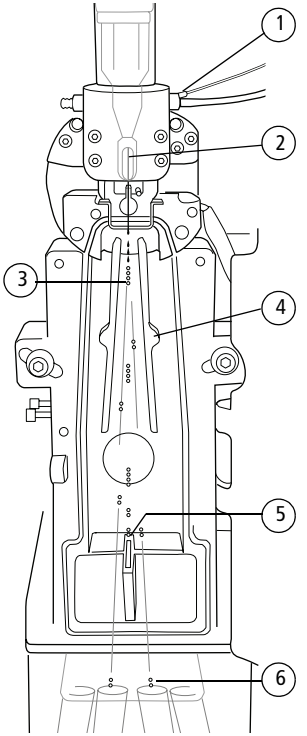
Sorting

During sorting, drop drive energy is applied to the stream to break it into highly uniform droplets. Droplets detach from the stream a few millimeters downstream from the nozzle. The time between when a particle intercepts the laser and when it reaches the droplet breakoff point is determined using BD FACS Accudrop technology (see Setting the Drop Delay on page 69).

When a particle is detected that meets the predefined sorting criteria, an electrical charge is applied to the stream just as the droplet containing that particle breaks off from the stream. Once broken off from the stream, the droplet—now surrounded by air—still retains its charge. The charged droplet passes by two strongly charged deflection plates. Electrostatic attraction and repulsion cause each charged droplet to be deflected to the left or right, depending on the droplet's charge polarity. Uncharged droplets are not affected by the electric field and pass down the center to the waste aspirator. See Figure 2-14 on page 63.

The following sections describe how the BD FACSAria instrument creates and charges drops, how the drops are deflected, and how sorting populations are identified.

Figure 2-14 Sorting



1. Charge is applied via the stream-charging wire in the barb.
2. Sample generates light scatter and fluorescent signal; signal is analyzed.
3. The charged droplet breaks off.
4. Deflection plates attract or repel charged droplet.
5. Uncharged droplets pass to waste.
6. Charged drops containing particles of interest are collected.

Drop Formation

The BD FACSAria instrument is unique in that drop drive energy is constantly applied to the stream; droplets form as soon as you turn on the stream. Sample interrogation takes place upstream of the stream vibration so that analysis is not affected by the drop drive.

A drop breakoff image is created using an LED strobe and a video camera. The image is displayed in the Breakoff window. (See Breakoff Window on page 64.) Patented Sweet Spot technology* analyzes the drop breakoff image and provides feedback to the appropriate instrument controls.

* US Patent No. 5,700,692

Breakoff Window

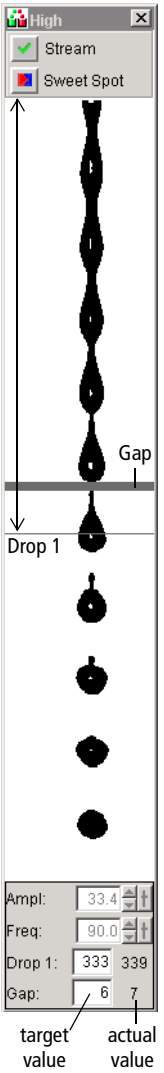
The upper camera transmits an image of the drop breakoff to the Breakoff window, where video image processing software converts the visual characteristics of the image into numerical properties. The drop breakoff is analyzed for two key features: Drop 1 and Gap.

Drop 1 is defined as the number of pixels from the top of the image to the center of gravity of the first broken-off drop. A thin gray line on the image is used to identify this drop. The number shown to the right of the Drop 1 field at the bottom of the window is the actual pixel location of the gray line. The number entered into the Drop 1 field is the user-defined Drop 1 target.

The Gap is defined as the number of pixels from the first discontinuity in the stream to the next stream object, generally the first broken-off drop. A gray line of varying thickness represents the Gap. The number shown to the right of the Gap field is the pixel width of the gray line. The number entered into the Gap field is a user-defined target.

When the Sweet Spot is turned on, the drop drive amplitude (Ampl.) is automatically adjusted to approximately match the target Drop 1 and Gap values. The amplitude is initially adjusted in larger increments until Drop 1 is achieved. The amplitude is then adjusted in smaller increments until the instrument attains the target Gap. The Sweet Spot feature will make adjustments as necessary to maintain the required breakoff conditions throughout the day.

The Sweet Spot performs two other functions during sorting. When sorting, if the Drop 1 or Gap are out of range, sorting is paused until the values are back within range. This ensures that sorting occurs only under the proper breakoff conditions. If a more severe problem such as a clog is detected by the Sweet Spot, the stream is shut off and sorting is stopped: the deflection plates shut off, the aspirator drawer closes, and the sample tube is unloaded.



Preprogrammed values can be downloaded to the Breakoff window by choosing High, Medium, Low, or Custom from the Sort > Sort Setup menu.

Note that changes to values in the Sort Setup windows (Breakoff and Side Stream) are automatically saved. At startup, the last settings used on the instrument are restored, except the Stream and Sweet Spot controls which always default to off. Default values can be restored by choosing Sort > Sort Setup > Restore Defaults. For more information, see Sort Setup on page 92.

Table 2-1 Breakoff window controls

Control	Description
Stream button	Turns the stream on and off by clicking
Sweet Spot button on off	Enables automatic adjustment of the drop drive amplitude to maintain the stability of the breakoff point When the Sweet Spot is on, the Amplitude and Frequency fields are disabled; the amplitude is automatically adjusted by the software. To enable the fields, turn off the Sweet Spot.
Amplitude field	Adjusts the amplitude or intensity of the drop drive, from 1.0–80.0 volts The drop drive amplitude determines the breakoff point. A higher amplitude value results in a shorter stream breakoff; a lower amplitude results in a longer stream breakoff. Typically, the amplitude is set once, at the beginning of a sorting experiment, and then maintained via the Sweet Spot.
Frequency field	Determines the number of drops formed per second and the size of the drops (Drop size is also influenced by the nozzle size.) The drop drive frequency can be adjusted from 1.0–102.0 kHz. The higher the frequency, the more drops are generated per second and the smaller the drops. The lower the frequency, the fewer drops generated per second and the larger the drops.

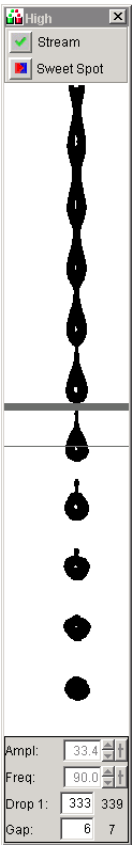


Table 2-1 Breakoff window controls (continued)

Control	Description
Frequency field (continued)	In general, the drop drive frequency should not need adjustment. BD Biosciences recommends using the default values that are input with each Sort Setup mode.
Drop 1 field	The distance between the top of the image and the center of the first broken-off drop, from 100–600 pixels When you input a value and turn on the Sweet Spot, the instrument automatically adjusts the amplitude to attain your target value. Note that not all Drop 1 targets are attainable. The Drop 1 value jumps in whole increments of approximately 57 pixels according to your drop spacing. The same Drop 1 setting should be used from day to day; a difference of up to 10 pixels between the target value and the actual value is acceptable.
Gap field	Gap between the stream breakoff and the top of the first drop The default Gap setting is 6 pixels, and in general, it should never be adjusted. Differences of 1–2 pixels between the target value and the instrument-generated value are acceptable.

Typically, when setting up for sorting, you use the Amplitude to set the required drop breakoff, and copy the generated Drop 1 value to the target field. Then, you turn on the Sweet Spot to maintain the drop breakoff. When the Sweet Spot detects a >1-pixel difference between the target Gap setting and the actual Gap, it adjusts the Amplitude to reduce the Gap. When a >2-pixel difference is detected, the Sweet Spot stops sorting temporarily (stops charging the stream) until the Gap is restored. Once the Gap is back within range, sorting automatically resumes.

☑ **Tip** To save screen space, you can hide the fields at the bottom of the window after the amplitude has been set. To do so, right-click the window and choose Hide Controls from the contextual menu. (You can also choose to hide button labels.) To show the controls again, right-click the window and choose Show Controls.

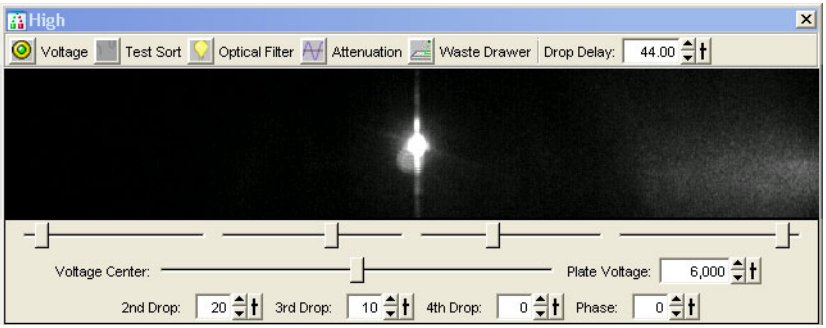
For information on setting the breakoff, see Setting Up the Breakoff on page 106.

Side Stream Formation

Side streams are formed when the voltage is on and you are sorting, or when you click Voltage, then Test Sort in the Side Stream window.

The Side Stream window displays an image of the side streams as transmitted by the lower camera. In addition to the stream image, the Side Stream window contains the controls used to adjust electrical charges and to determine the drop delay using Accudrop, as described in Setting the Drop Delay on page 69.

Figure 2-15 Side Stream window



Controls in the Side Stream window are described in Table 2-2 on page 68. Preprogrammed values can be downloaded to the Side Stream window by choosing High, Medium, Low, or Custom from the Sort > Sort Setup menu.

Note that changes to values in the Sort Setup windows (Side Stream and Breakoff) are automatically saved. At startup, the last settings used on the instrument are restored, except the states of the Voltage, Test Sort, and Optical Filter buttons, which always default to off. Default values can be restored by choosing Sort > Sort Setup > Restore Defaults. For more information, see Sort Setup on page 92.

☑ **Tip** To save screen space, you can hide the controls in the lower half of the window. To do so, right-click the window and choose Hide Controls from the contextual menu. (You can also choose to hide button labels.) To show the controls again, right-click the window and choose Show Controls.

Table 2-2 Side Stream window controls

Control	Description
Voltage button	Turns the plate voltage on and off by clicking
Test Sort button on off	Generates test side streams based on test sort pulses when the button is clicked
Optical Filter button in out	Controls the position of the optical filter in front of the lower (Accudrop) camera
Attenuation button on off	Decreases the amplitude of the drop drive when the button is clicked. At startup, attenuation defaults to off. As a general rule, turn on attenuation when sorting below 30 psi.
Waste Drawer button closed open	Moves the aspirator drawer in or out depending on its current state. The default state is in. For more information, see Aspirator Drawer on page 33.
Drop Delay field	Sets the amount of time between when an event is measured and the breakoff point, from 10–140 drops. The drop delay value determines which drop will be deflected. The drop delay value is set experimentally using BD FACS Accudrop technology.
Voltage sliders (far left, left, right, far right)	Set the percentage of charge to be applied to the corresponding stream (as a percentage of maximum)
Voltage Center slider	Adjusts the relative voltage between the left and right plates which moves the streams slightly to the left or right as a whole
Plate Voltage field	Adjusts the total voltage difference between the plates, which determines the angle of stream deflection

Table 2-2 Side Stream window controls

Control	Description
2nd, 3rd, 4th Drop fields	Apply a correction factor for the drop charge as a percentage of the previous drop, from –100 to 100%
Phase field	Adjusts the phase between drop generation and charging of the droplets from 0–360 degrees The selected value is sent to both the drop-charging electrode and the drop strobe. In general, the Phase never needs adjusting; you can keep the default value of zero.

Setting the Drop Delay

The BD FACSAria instrument includes integrated Accudrop technology to assist in setting an accurate drop delay value. Accudrop components consist of the following:

- a diode laser, mounted to the left of the sort block
- a camera that provides an image of the side streams
- an emission filter for viewing the fluorescence from Accudrop beads

The emission filter is installed in front of the lower camera and can be moved in and out by clicking the Optical Filter control. When the button is yellow ( ), the filter is out. This position is used to view the center and side streams.

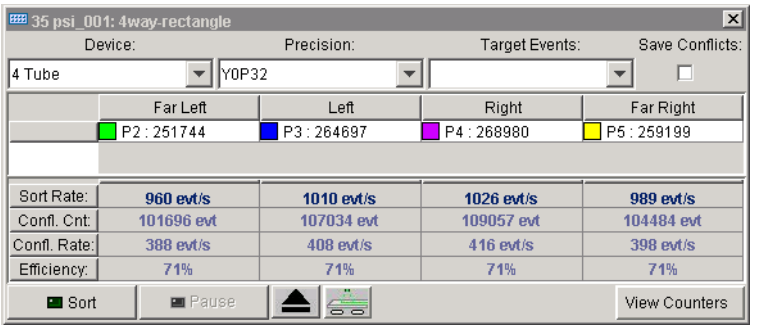
Click the button to move the filter in front of the camera when you are determining the drop delay; the button changes to red ( ). To determine the drop delay, the streams are illuminated by the diode laser just below the point of deflection. Specialized fluorescent particles (Accudrop beads) can be viewed in the center and left side streams as the delay is adjusted. The best delay yields the most particles in the left stream and the fewest in the center stream. See Determining the Drop Delay on page 154 for more information.

Drop Charging

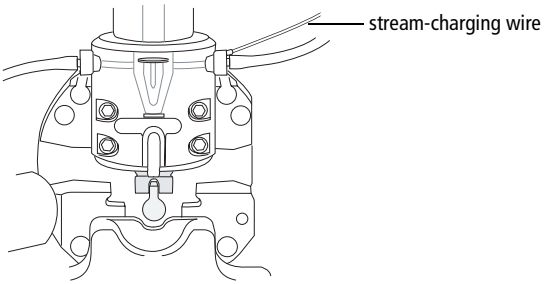
Drops are charged when an event is detected that meets the defined sorting criteria, as determined by the Sort Layout window.

The Sort Layout is a floating window containing all sorting instructions and controls (Figure 2-16). The sort layout designates which device will be used to collect sorted particles and which particles will be sorted into each sort location. Up to four sort counters can be displayed in the window to give ongoing status during a sort. See Sort Layout on page 94 for more information about the Sort Layout window.

Figure 2-16 Sort layout for four-way sort



Target events are identified by drawing gates around populations of interest in plots. The Sort Layout window specifies which gated population should be sorted into each sort collection tube or spot in a plate or on a slide. During sorting, when an event is identified within one of the sort gates, the drop containing the particle of interest is charged via the stream-charging wire in the nozzle.

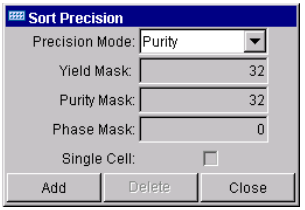


The amount and type of charge determines where the drop will be sorted. For example, in a four-way sort, drops with the most charge will be deflected into the outer streams, while drops with less charge will be deflected into the inner streams.

Conflict Resolution During Sorting

During sorting, the cytometer deflects drops based on the characteristics of the particles in each drop and where the user wants to deflect them. Conflicts can occur depending on the type of target particle, where the particle is located within a drop, or whether the drop is free of contaminating particles.
BD FACSDiva software accurately measures particle position to within 1/32 of a drop.

Mask settings determine how drops are deflected when sorting conflicts occur. There are three Mask settings, each of which addresses a different type of conflict. These settings are combined to define sort *Precision Modes*: each mode is made up of a set of masks. Precision Modes are defined in the Sort Precision dialog box, accessed from the Sort menu.

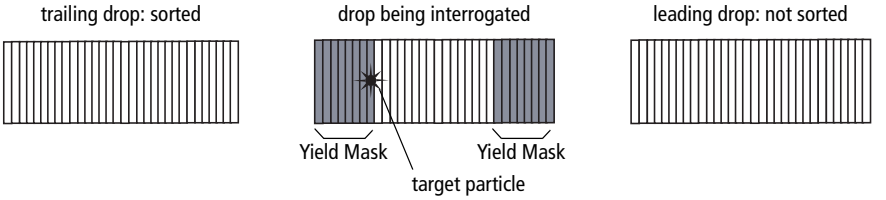


Yield Mask

The *Yield Mask* setting defines how close to the edge of the drop, in 1/32-drop increments, a particle of interest can be located before sorting an additional drop. Half of each Yield Mask setting defines an equal area at each end of the drop.

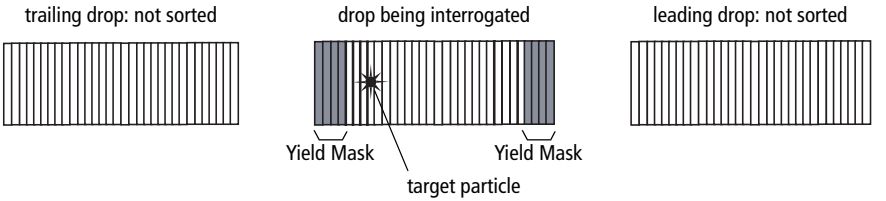
For example, when the Yield Mask is set to 16 and an event is within 8/32 from the beginning of a drop, the previous (leading) drop will be sorted. If an event is within 8/32 from the end of a drop, the following (trailing) drop will be sorted. See Figure 2-17 on page 72.

Figure 2-17 Target particle within a Yield Mask of 16



If the Yield Mask were set to 8 for the same target particle, the target particle would fall outside of the Yield Mask; thus no additional drops would be sorted. See Figure 2-18.

Figure 2-18 Target particle outside a Yield Mask of 8



When the Yield Mask is set to zero, only one drop (the drop containing the target particle) will be deflected; when the mask is set to 32, two drops will always be deflected. Yield Masks between 0–32 will sort either one or two drops.

NOTICE When more than one drop is deflected in the same direction, residual charge from the first drop degrades the quality of the side streams. Thus, when four-way sorting or sorting into small wells where precise deflection is required, enter zero for the Yield Mask.

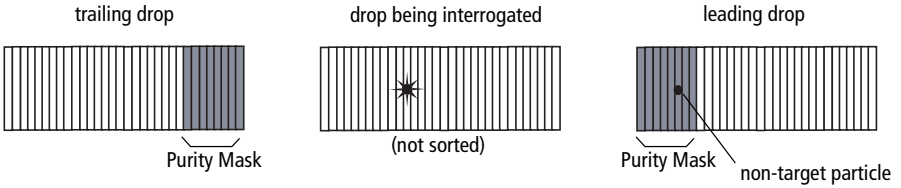
Yield Masks cannot be used in conjunction with Phase Masks. Thus, when the Yield Mask is greater than zero, the Phase Mask automatically reverts to zero.

Purity Mask

The *Purity Mask* setting defines how close, in 1/32-drop increments, a contaminating drop can be located before ignoring the drop being interrogated.

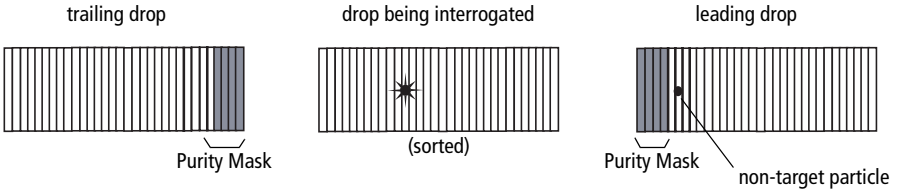
For example, when the Purity Mask is set to 16, the drop being interrogated will not be sorted if a non-target particle falls within the first or last 8/32 of the leading or trailing drop. In the following example, a non-target particle falls within the first 8/32, so the interrogated drop will not be sorted (Figure 2-19).

Figure 2-19 Non-target particle within a Purity Mask of 16



If the Purity Mask were set to 8 for the same target particle, the non-target particle would fall outside of the Purity Mask, so the interrogated drop would be sorted. See Figure 2-20.

Figure 2-20 Non-target particle outside a Purity Mask of 8



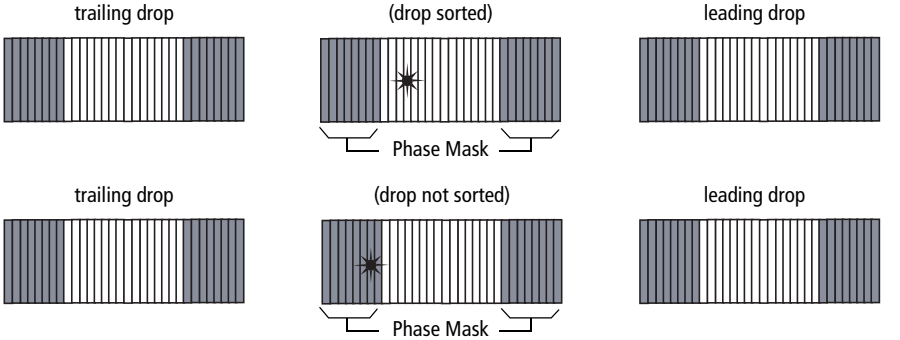
NOTICE With any Purity Mask greater than zero, the drop being interrogated must be free of contaminating particles or the drop will not be sorted.

Phase Mask

Particles near the drop edge can affect the breakoff and alter the trajectory of the deflected drop. The *Phase Mask* restricts drop deflection when an event is too close to the edge of a drop or when there are events close to the edge of adjacent drops. A Phase Mask is used to improve counting accuracy and side-stream quality at the expense of yield.

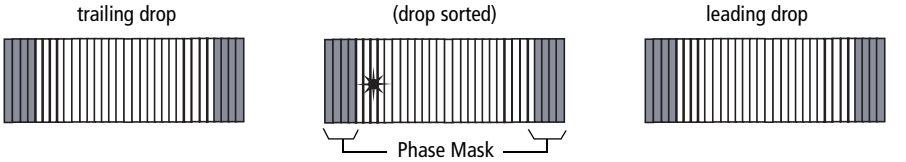
For example, when the Phase Mask is set to 16, the drop being interrogated will be sorted only if the target particle falls outside the Phase Mask (Figure 2-21).

Figure 2-21 Sorted and unsorted drop with Phase Mask of 16



Decreasing the Phase Mask to 8 allows more drops to be sorted. However, because the target particle is closer to the edge of the drop, there is more variability in drop trajectory (Figure 2-22).

Figure 2-22 Sorted drop with Phase Mask of 8

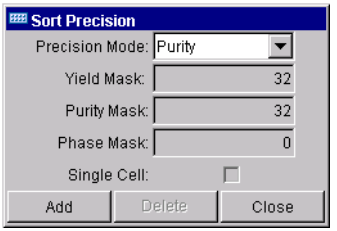


☑ **Tip** BD recommends using a Phase Mask of at least 8 when sorting single cells.

Phase Masks cannot be used in conjunction with Yield Masks. Thus, when the Phase Mask is greater than zero, the Yield Mask automatically reverts to zero.

Sort Precision Modes

Mask values can be combined in many different ways. By default, six Sort Precision modes are already defined—Purity, 4-Way Purity, Yield, Single Cell, Initial, and Fine Tune.



	Precision Mode					
	Purity	**4-Way Purity**	**Yield**	**Single Cell**	**Initial**	**Fine Tune**
Yield Mask:	32	0	32	0	32	0
Purity Mask:	32	32	0	32	0	0
Phase Mask:	0	0	0	16	0	0
Single Cell:	☐	☐	☐	☒	☐	☐

- In Purity mode, the Yield Mask is set to the maximum to obtain the greatest number of particles; because the Purity Mask is also set to the maximum, only drops free of contaminating particles will be sorted. Sorting in Purity mode results in a sorted sample that is highly pure, at the expense of recovery and yield.

- In 4-Way Purity mode, the Purity Mask is set to the maximum, so only drops free of contaminating particles will be sorted. The Yield Mask is set to zero to ensure that residual charges from adjoining drops do not degrade the quality of side streams. The 4-Way Purity mode is recommended for four-way sorting where precise deflection is required.

- In Yield mode, only the Yield Mask is used at its maximum value; thus recovery and yield are optimized at the expense of purity.

- In Single Cell mode, the Purity Mask is set to the maximum, so only drops free of contaminating particles will be sorted. The Phase Mask is set to half the maximum, so only particles centered within the sorted drop are deflected. Drop trajectory and count accuracy are optimized at the expense of yield. This mode is recommended for single-cell sorting or situations where precise counting is required.

☑ **Tip** Select the Single Cell checkbox to obtain the highest quality side streams and the most accurate counts. When the checkbox is selected, drops containing two target events (acceptable with a Purity Mask) are discarded. The Yield Mask is disabled when Single Cell is selected.

The remaining modes are used mainly during drop delay determination. Initial mode is equivalent to the Yield mode, but it is named differently as a reminder to use this as the initial mode when using Accudrop to set the drop delay.

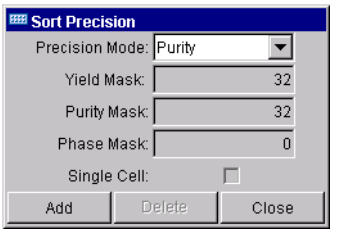
- In Initial mode, only the Yield Mask is used at its maximum value; thus recovery and yield are optimized at the expense of purity.

- In Fine Tune mode, all masks are set to zero to deflect the maximum number of drops. This mode is used to fine-tune the drop delay value. See Determining the Drop Delay on page 154 for more information.

Defining New Precision Modes

Default Precision modes cannot be edited; however, you can create new modes and then choose them from the Precision Mode drop-down menu.

1 Choose Sort > Sort Precision; click Add.



The current sort mode is duplicated and the Mask fields are enabled.

2 (Optional) Change the name of the mode in the Precision Mode field.

3 Enter values for the Yield, Purity, and Phase Masks.

4 Click to select the Single Cell checkbox, if needed.

5 Click Close.

The new mode is added to the Precision Mode drop-down menu.

To delete a mode, choose it from the drop-down menu and then click Delete.

3

Using BD FACSDiva Software

All BD FACSAria instrument functions are controlled using BD FACSDiva software. This chapter provides a general overview of the workspace components and describes software controls that are unique to the BD FACSAria instrument. For an in-depth description of software components not described in this chapter, refer to the *BD FACSDiva Software Reference Manual*.

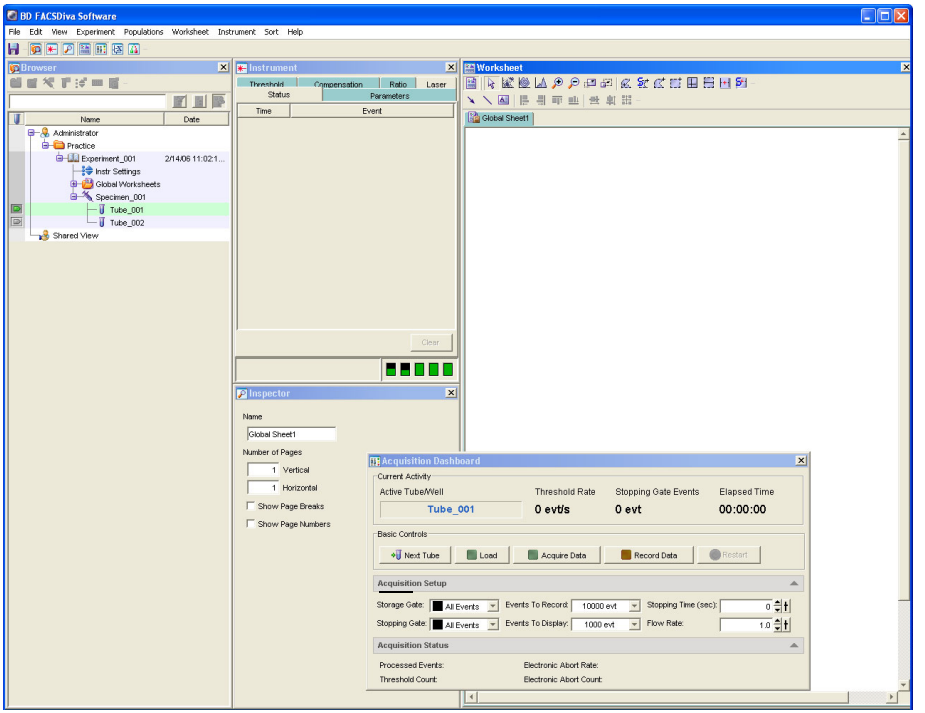
The following topics are covered in this chapter:

- Workspace Components on page 80
- Instrument Controls on page 81
- Sorting Controls on page 90
- Templates on page 102

Workspace Components

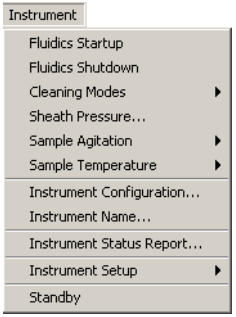
When you start BD FACSDiva software, the workspace appears (Figure 3-1). For a general overview of the workspace and to get started using the software, refer to *Getting Started with BD FACSDiva Software*. When running BD FACSDiva with the BD FACSAria, two additional windows can be displayed by clicking the Sorting button in the Workspace toolbar; see Sorting Controls on page 90 for a description.

Figure 3-1 BD FACSDiva workspace



Instrument Controls

Most BD FACSAria-specific instrument controls are accessed through the Instrument menu. Instrument controls on the Instrument menu are described in the following sections:



- Fluidics Controls on page 81
- Instrument Configuration on page 85
- Instrument Status Report on page 88

Other menu commands (Instrument Name, Instrument Setup) are described in the *BD FACSDiva Software Reference Manual*. Additional instrument controls are located in the Acquisition Dashboard; see Acquisition Controls on page 89.

Fluidics Controls

Fluidics control of the BD FACSAria instrument is completely automated by BD FACSDiva software. The software contains pre-programmed fluidics protocols that are activated by choosing the corresponding menu command from the Instrument menu. In addition, fluidics level indicators are available in the Instrument window; see Fluidics Level Indicators on page 84.

Fluidics Startup

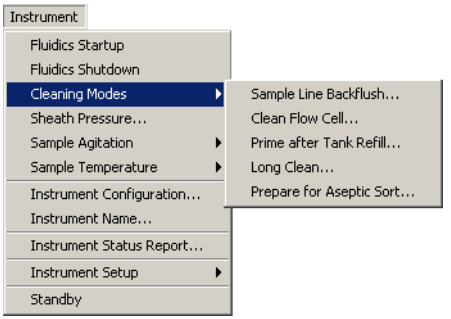
During fluidics startup, waste and sheath levels are verified and the fluidics system is primed with sheath solution. See Instrument Startup on page 104 for more information.

Fluidics Shutdown

Fluidics shutdown removes sheath from the lines and plenum and replaces it with DI water, and cleans the cuvette with a cleaning solution. See Fluidics Shutdown on page 172 for more details.

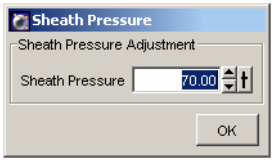
Cleaning Modes

BD FACSDiva software contains pre-programmed cleaning modes that are activated by choosing the corresponding menu command from the Instrument > Cleaning Modes menu. See Internal Cleaning on page 175 for more information.



Sheath Pressure

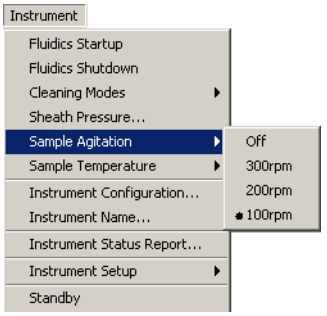
The sheath pressure determines how quickly particles pass through the laser beam. Choose Instrument > Sheath Pressure to access a dialog box where you can view the current sheath pressure.



Each sort setup option is optimized at a preset sheath pressure. If you change the sheath pressure, a multitude of other values will be affected, including the drop drive frequency, drop charge levels, laser delay, area scaling factor, and other values. For proper instrument operation, change the sheath pressure by choosing an option from the Sort > Sort Setup menu; do not adjust the pressure using controls in the Sheath Pressure dialog box. Note that the Sheath Pressure command is disabled when the Sweet Spot is on.

Sample Agitation

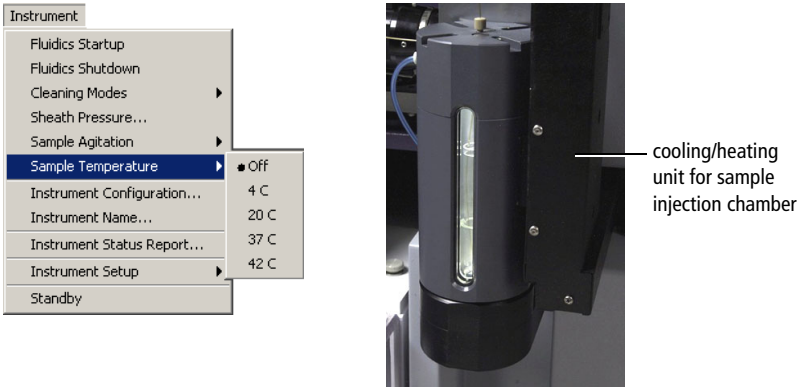
Choose Instrument > Sample Agitation to specify the speed at which samples are agitated. You can choose from one of the specified values, or choose Off to turn off agitation.



Sample Temperature

Use the Sample Temperature command to set the temperature inside the sample injection chamber. You can choose from one of the specified values, or choose Off to turn off temperature control (Figure 3-2).

Figure 3-2 Setting the sample temperature



NOTICE The cooling/heating unit is designed to maintain the temperature of a sample tube; it is not designed to cool or heat the sample. It takes approximately

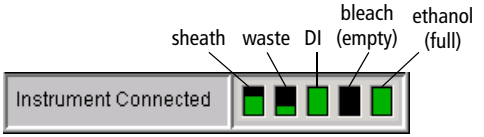
45 minutes to reach the required temperature inside the chamber, during which time the chamber must be kept closed.

To cool or heat the chamber, install a sample tube and click Load. Click Stop Acquiring to keep the chamber closed and stop running the sample. To maintain the temperature, do not leave the chamber open for extended periods while changing sample tubes.

Fluidics Level Indicators

BD FACSDiva software provides fluidics level indicators in the Instrument window (Figure 3-3). The sheath and waste indicators provide an approximate indication of the fluid levels in each tank. The DI, bleach, and ethanol tank indicators appear full until the fluid level descends below 20% of the tank capacity. When this occurs, the corresponding level indicator changes to black.

Figure 3-3 Levels indicators



When the sheath is low or the waste is full while the stream is running, the corresponding indicator turns red and the following warning message is displayed every 5 minutes until the stream is turned off. If the warning message is not dismissed, the system automatically turns off the stream after 15 minutes.

Do not dismiss the warning message without refilling the sheath or emptying the waste. If you continue to run the instrument after dismissing the message, the waste container could overflow or the sheath container could run dry.

If the instrument is run until the sheath supply is depleted, air will accumulate in the bubble filter. Purge the filter as described in Purging the Bubble Filter on page 208. If you are unable to remove all of the air, replace the bubble filter as described in Changing the Bubble Filter on page 197.

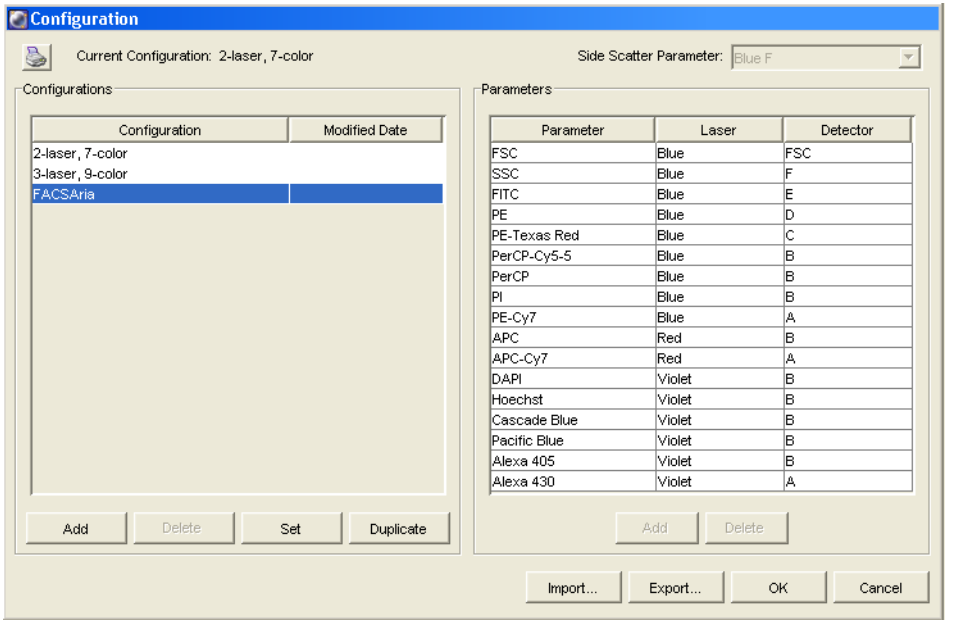
For a description of other controls in the Instrument window, refer to the *BD FACSDiva Software Reference Manual*. Delay information specific to the BD FACSAria instrument is described in Laser Delay on page 61.

Instrument Configuration

The BD FACSAria instrument is equipped with a specific set of lasers, filters, and dichroic mirrors. The Instrument Configuration dialog box lets you define which fluorochromes or cell parameters will be measured at each photomultiplier tube (PMT) detector. Within this dialog box, you can define parameters for an unlimited number of fluorochromes, up to three lasers, and up to 15 detectors.

The following default configurations are provided when you install BD FACSDiva software for the BD FACSAria instrument (Figure 3-4 on page 86). The FACSAria configuration contains the parameters shown in Table 1-1 on page 40.

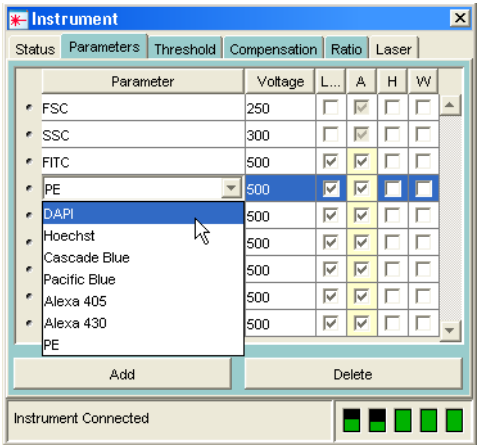
Figure 3-4 Default configuration



Before you start any experiment, verify that the instrument configuration contains appropriate parameters for the samples you are running and that the instrument optics match the current configuration. See Application Options on page 242. If needed, you can add or modify parameters in an existing configuration or define a custom configuration, as described in Defining a Custom Configuration on page 245.

For accurate data results, the instrument optics must match the current instrument configuration.

Selections in the instrument configuration determine which parameters are available for your experiment. When more than one parameter is available for a detector, the first parameter is listed by default. To choose a different parameter, click the name in the Parameters tab and choose a different option from the drop-down menu:



☑ **Tip** To ensure that your experiment's parameters match those in the current instrument configuration, always record data with the same configuration.

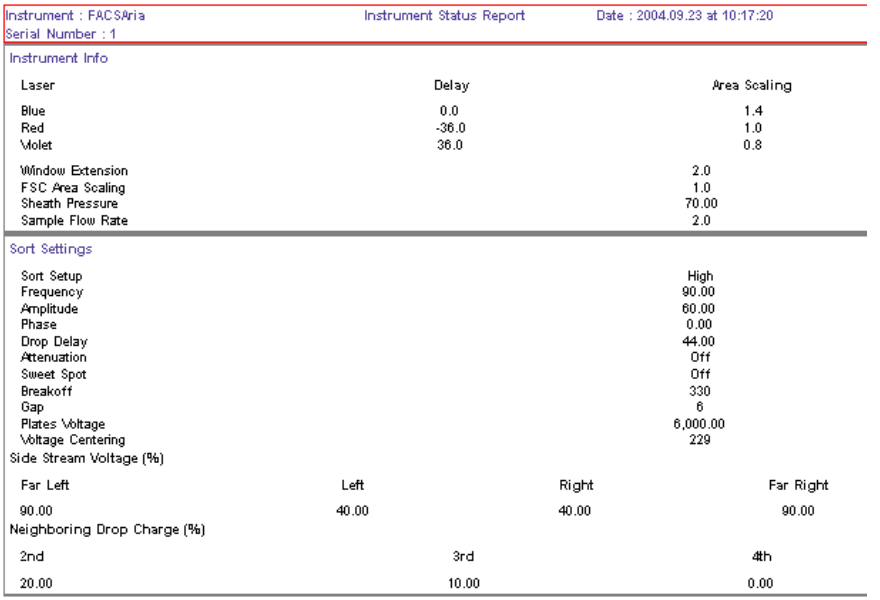
Instrument Status Report

Choose Instrument > Instrument Status Report to view a report of the current instrument settings. The Instrument Status Report is displayed in a separate window with a menu bar above the report header.

For a full description of the Instrument Status Report, refer to the *BD FACSDiva Software Reference Manual*. A BD FACSAria instrument report includes the following additional information.

- The Instrument Info section lists the sheath pressure and flow rate, along with the lasers used, corresponding Laser Delay and Area Scaling factors, and the Window Extension.

- The Sort Settings section lists all Sort Setup values, along with the Plate Voltage and Voltage Center values. If the Sweet Spot is off, Breakoff and Gap values are shown; if the Sweet Spot is on, Drop 1 and target Gap values are shown.



Instrument : FACSAria — Instrument Status Report — Date : 2004.09.23 at 10:17:20
Serial Number : 1

Instrument Info

Laser	Delay	Area Scaling
Blue	0.0	1.4
Red	-36.0	1.0
Violet	36.0	0.8

Window Extension	2.0
FSC Area Scaling	1.0
Sheath Pressure	70.00
Sample Flow Rate	2.0

Sort Settings

Sort Setup	High
Frequency	90.00
Amplitude	60.00
Phase	0.00
Drop Delay	44.00
Attenuation	Off
Sweet Spot	Off
Breakoff	330
Gap	6
Plates Voltage	6,000.00
Voltage Centering	229

Side Stream Voltage (%)

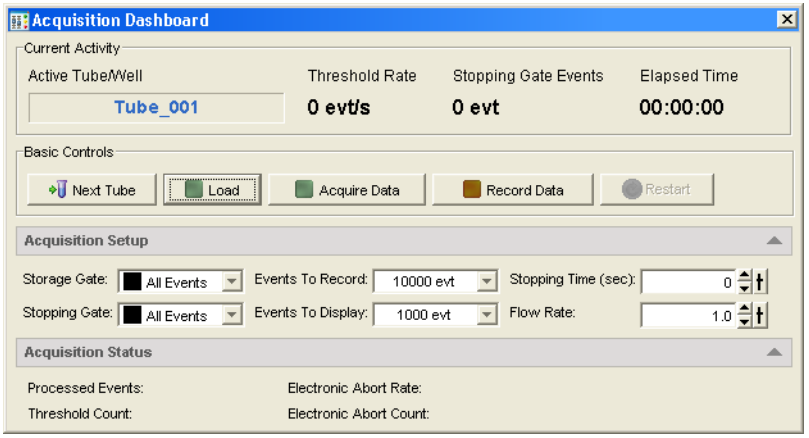
Far Left	Left	Right	Far Right
90.00	40.00	40.00	90.00

Neighboring Drop Charge (%)

2nd	3rd	4th
20.00	10.00	0.00

Acquisition Controls

Along with the controls described in the *BD FACSDiva Software Reference Manual*, the following acquisition controls are available for the BD FACSAria instrument.



- Load—lifts a tube into the sample injection chamber, starts sample agitation (if agitation is turned on), and starts acquisition of the sample

  When a tube is already loaded, the button changes to Unload. Clicking Unload stops acquisition of the sample, turns off the agitator, and lowers the tube from the sample injection chamber.

  NOTICE The Load button is enabled only when the workstation is connected to the instrument, an experiment is open, and the stream is turned on.

- Stop Acquiring—stops sample acquisition without unloading the sample tube

  The Acquire Data button functions only when a tube is loaded.

- Flow Rate—controls the sample flow rate, from 1.0–11.0 (10–120 µL/min)

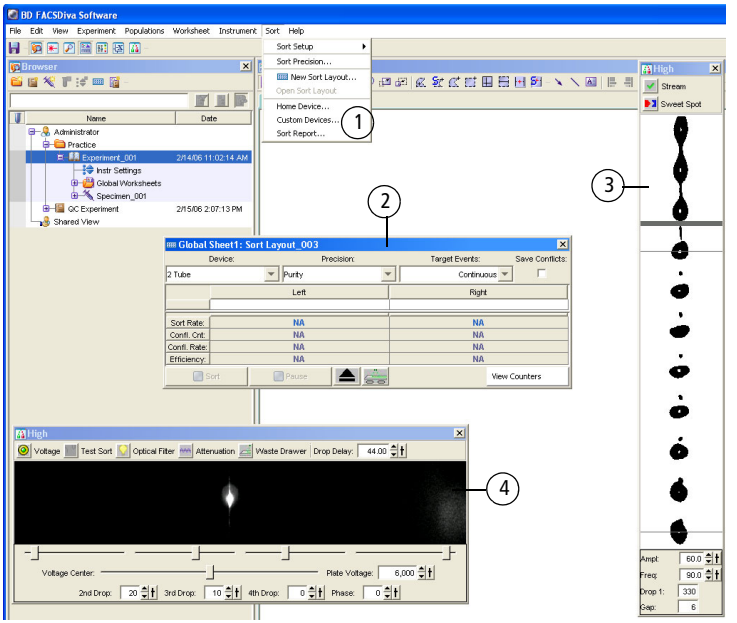
  Do one of the following to change the flow rate:

  - Select the value in the field and enter a new value.
  - Click the pointer in the slider bar and drag it to a new value.
  - Use the mouse to click the up and down arrows or press the arrow keys on your keyboard to increase or decrease the values in small increments.

Sorting Controls

All sorting on the BD FACSAria instrument is controlled by BD FACSDiva software. Sorting controls are shown in Figure 3-5.

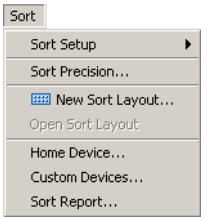
Figure 3-5 BD FACSDiva sorting controls



①	The **Sort menu** provides access to sort setup and sort precision modes, sort layouts, Sort Reports, and sort devices used with the automated cell deposition unit (ACDU). See Sort Menu on page 91.
②	The **Sort Layout** window designates which device will be used to collect sorted particles and which particles will be sorted into each sort location. Sorting controls and counters are also displayed in the window. See Sort Layout on page 94.
③	The **Breakoff** window displays an image of the stream and contains controls to adjust drop formation. See Drop Formation on page 63.
④	The **Side Stream** window displays an image of the side streams, and contains controls to adjust electrical charges and the drop delay. See Side Stream Formation on page 67.

Sort Menu

Choose commands in the Sort menu for the following.



- Sort Setup—downloads the most recently used settings for high-, medium-, or low-pressure sorting; see Sort Setup on page 92.
- Sort Precision—opens a dialog box where you can choose or define a Sort Precision mode for handling sorting conflicts; see Conflict Resolution During Sorting on page 71.
- New Sort Layout—opens the default 2-Tube Sort Layout window where other sort layouts can be chosen; see Sort Layout on page 94. (Note that clicking the Sort Layout button on the Browser toolbar performs the same function.)
- Open Sort Layout—opens an existing sort layout. A sort layout must be selected in the Browser for this menu command to be enabled. Alternatively, double-click any sort layout to open it.
- Home Device—opens a dialog box containing commands to move the tray support arm, either manually or to the home position. See Setting Up the Stream on page 165.

- Custom Devices—opens a dialog box where custom devices can be defined. See Creating a Custom Device on page 168.

- Sort Report—displays a report showing the sort settings, acquisition counters, and sort layout information from the current sort. See Sort Report on page 100.

Sort Setup

Sort setup values for high-, medium-, and low-pressure sorting can be downloaded using the Sort > Sort Setup command. Default settings are provided for each sorting option, along with custom settings for non-sorting applications. Use the following guidelines to determine which option to use.

Sort Setup	Nozzle (μm)	Recommended Applications
High	70	High-speed, high-throughput sorting
Medium	70	Sorting of cell lines, fragile cells
Low	100	Single-cell sorting, ACDU sorting, sorting of large or fragile cells
Custom	70	Non-sorting mode for high sensitivity

If you make changes to any of the settings, changes are automatically saved when you quit BD FACSDiva software or when you switch to a different sort setup mode. When you restart, the most recently used set of values is restored. You can restore default High, Medium, or Low settings by choosing Sort > Sort Setup > Restore Defaults. Default settings are shown in Table 3-1 on page 93.

Table 3-1 Default Sort Setup values

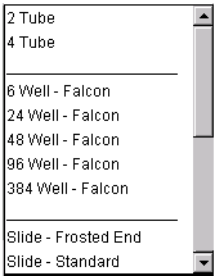
Setting	High	Medium	Low	Custom
Sheath Pressure	70	35	20	20
Amplitude	60	50	20	10
Frequency	87	60	30	30
Drop 1	330	330	330	330
Gap	6	6	6	6
Attenuation	off	on	on	off
Drop Delay	44.00	45.00	29.00	10.00
Far left voltage	90	45	45	0
Left voltage	40	20	20	0
Right voltage	40	20	20	0
Far right voltage	90	45	45	0
Plate voltage	6,000	5,000	5,000	0
2nd Drop	20	20	10	0
3rd Drop	10	10	5	0
4th Drop	0	0	0	0
Laser Delay (blue)	0.00	0.00	0.00	0.00
Laser Delay (red)	-36.00	-54.00	-40.00	-82.00
Laser Delay (violet)	36.00	54.00	40.00	82.00
Area Scaling (blue)	1.40	1.20	1.40	0.75
Area Scaling (red)	1.00	0.75	1.00	0.50
Area Scaling (violet)	0.80	0.35	0.50	0.25
Window Extension	2.00	2.00	2.00	4.00

Sort Layout

A sort layout is a floating window containing all sorting instructions and controls. The sort layout designates which device will be used to collect sorted particles and which particles will be sorted into each sort location. Up to four sort counters can be displayed in the window to give ongoing status during a sort.

Only one sort layout can be open at a time, but you can create several layouts for a single tube, as long as each sort layout has a different name. Sort layouts can also be added to global worksheets.

Sort layouts are available for up to nine default collection devices; additional custom devices can be defined. See Creating a Custom Device on page 168.



default collection devices

Examples of sort layouts for different devices are shown in the following figures; instructions for setting up the sort layout can be found in Setting Up a Sort Layout on page 96.

Figure 3-6 Sort layout for collection tubes (top) and for 48-well plate (bottom)

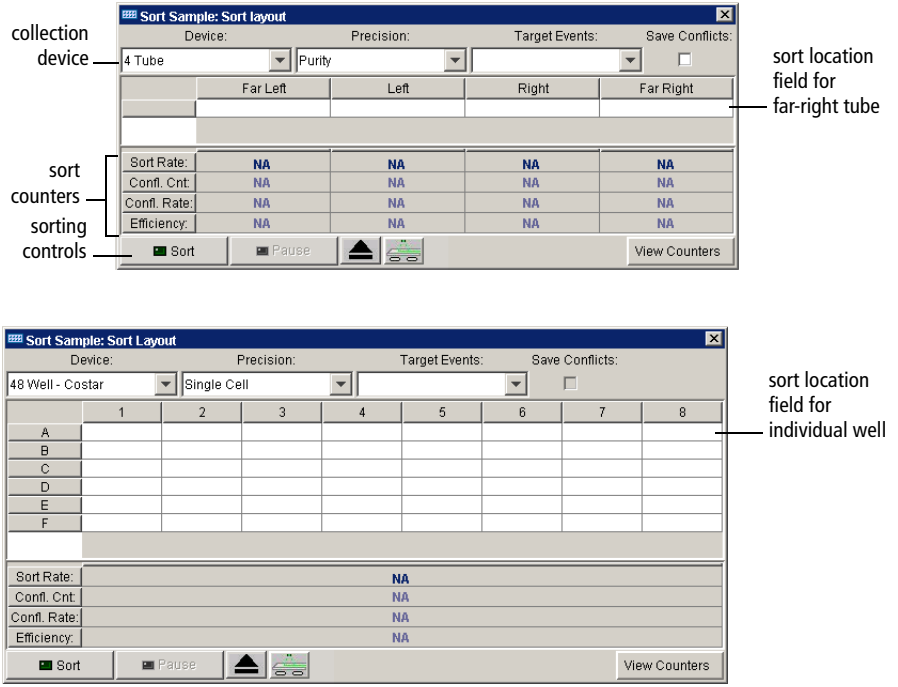
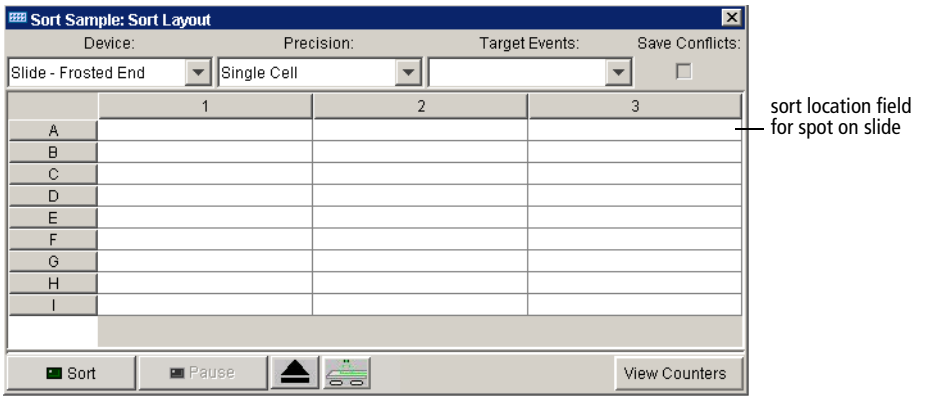


Figure 3-7 Sort layout for frosted slide



Setting Up a Sort Layout

Sort layouts can be added to tubes or global worksheets.

- Create tube-specific layouts if you are sorting different populations or using different sort devices for each tube in the experiment.
- Create global worksheet–specific layouts if you are sorting the same populations into the same sort device for all tubes in the experiment.

NOTICE To create a tube-specific sort layout, make sure a normal worksheet (gray-tinted tab) is shown in the Worksheet window *before* you create the layout.

1. Select the icon for a tube or global worksheet in an open experiment, and click the New Sort Layout button in the Browser toolbar ( ).
2. In the Sort Layout window, choose the type of device from the Device menu.

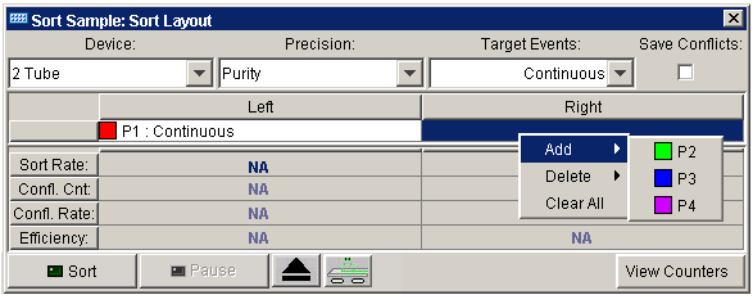
   Default sorting devices are listed along with any defined custom devices. The Sort Layout window changes depending on the selected device: the number of rows and columns in the window matches the number of tubes, wells, or spots in the collection device.
3. Choose the sort precision mode from the Precision menu.

   For more information, see Sort Precision Modes on page 75.
4. Enter the number of events to be sorted in the Target Events field.

   Once defined, the number of events can be reused by choosing from the drop-down menu. For continuous sorting, choose Continuous from the Target Events menu.
5. Select the field(s) corresponding to the tube(s), well(s), or spot(s) where the population will be sorted and choose a defined population from the Add menu.

   After you click in a sort location field, a menu appears where you can choose to add, delete, or clear all populations in the field (Figure 3-8).

Figure 3-8 Adding populations to be sorted



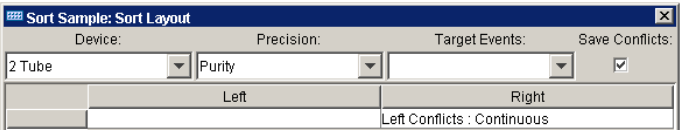
After you add a population, the population and the number of target events are written to the corresponding sort location field.

☑ **Tip** Select a row or column header to select all fields in that row or column. After adding a population, it will be written to all selected fields at once.

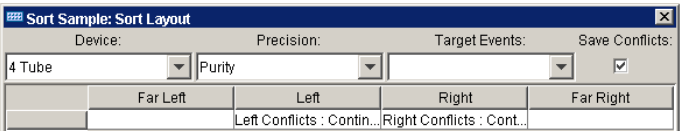
6 Specify whether to save sort conflicts by selecting the Save Conflicts checkbox.

This checkbox is enabled only when using a two- or four-tube layout. When selected, all sort conflicts are sorted into a default location.

- For a two-tube layout, conflicts are sorted to the right; no other populations can be sorted to the right-most tube.



- For a four-tube layout, conflicts for the Far Left tube are sorted to the left; conflicts for the Far Right tube are sorted to the right. No other populations can be sorted into the center-most tubes.



Editing a Sort Layout

- To change the number of events for any population, click on the Sort Location field(s) containing the population, and then choose a number from the Target Events field or enter a new number.

- To remove a population from a sort location field, select the field, and then choose the corresponding population from the Delete menu.

- To clear all populations from a field, select the field, and then choose Clear All.

Using Sorting Controls

Sorting controls appear at the bottom of the Sort Layout window. Use these controls for the following functions.

- Sort—starts sorting events for the current acquisition tube. All counters reset to zero when this button is clicked. Events are sorted until the requested number of sorted events has been reached.

  Click the Sort button again to stop sorting before reaching the requested number of events; the counters stop at the number of sorted events. If you click Sort to restart sorting, the counters reset to zero.

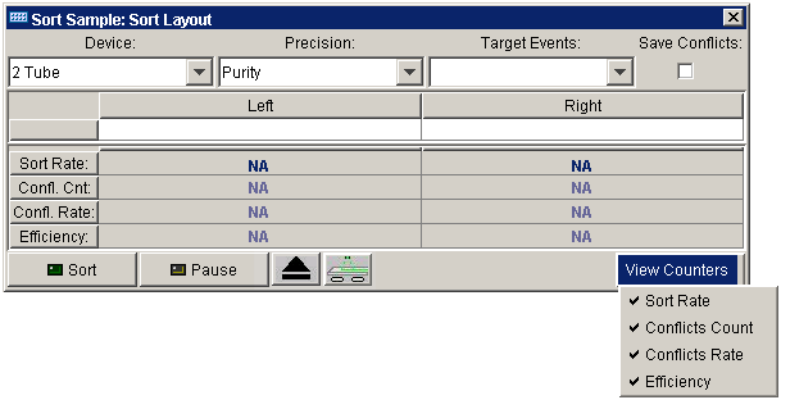
- Pause—stops sorting, but not acquisition; sort counters freeze when the Pause button is clicked. Click the Pause button again to continue sorting and to continue incrementing the sort counters.

- Access Stage—when the ACDU stage is in the back, brings the stage forward to put a plate on or take a plate off the stage. When the stage is in the front, sends the stage to the back and out of the way so you can install a collection tube holder.

- Move Drawer—moves the aspirator drawer in or out depending on its current state. The default state is in. For more information, see Aspirator Drawer on page 33.

Using Counters

Counters provide ongoing status during sorting; the fields cannot be edited. To display fewer counters in the Sort Layout window, click the View Counters button and choose a menu option. The corresponding counter is hidden. (Only counters with a checkmark next to the name are displayed.)



Counters display the following information:

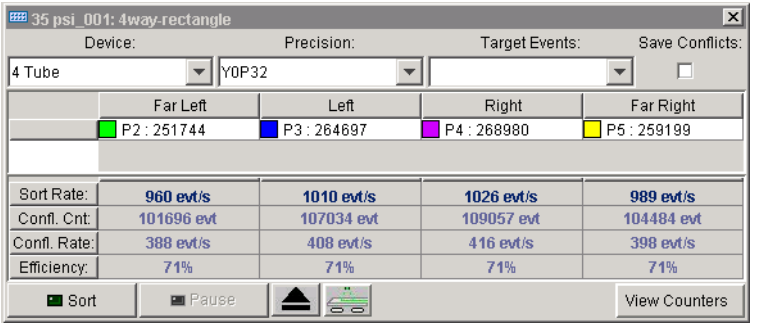
- Sort Rate—number of events/second that met the sort criteria and were sorted
- Conflict Count—number of events that met the sort criteria but were not sorted because of conflicts
- Conflict Rate—number of conflicts/second
- Efficiency—number of sorted events/(sort conflicts + sorted events) x 100

Monitoring a Sort

During sorting, each sort location field displays the number of actual sorted events. When a target number is specified, the field displays the actual number of events along with the number of target events (Figure 3-9).

A progress bar appears behind the Sort Rate counter field showing the progress of the sort.

Figure 3-9 Sort Layout during sorting



Sort Report

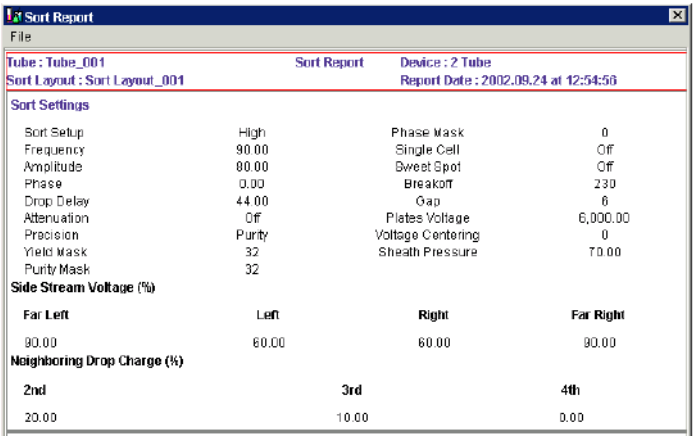
Choose Sort > Sort Report to view a report of the current sort layout. This menu item is enabled only if a sort layout is open and the instrument is not sorting. The Sort Report can be printed or exported.

☑ **Tip** To save counter information, print a Sort Report immediately after sorting. When you close the sort layout, all counter information is lost.

A Sort Report contains the following:

- Header information—tube name, sort layout name, type of collection device, and the date and time of printing
- Sort settings—sort setup values, precision mode, and masks definition

- Acquisition counters—threshold count, processed events count, electronic conflicts count, and elapsed time
- Sort counters—counter values per sort destination, or total sort count if sorting sequentially
- Sort Layout—population(s), sort count, and target event count for each sort location field



The Sort Report window contains a File menu where you can choose to print or export the report. Exported comma-separated value (CSV) files can be opened with a spreadsheet application such as Microsoft Excel.

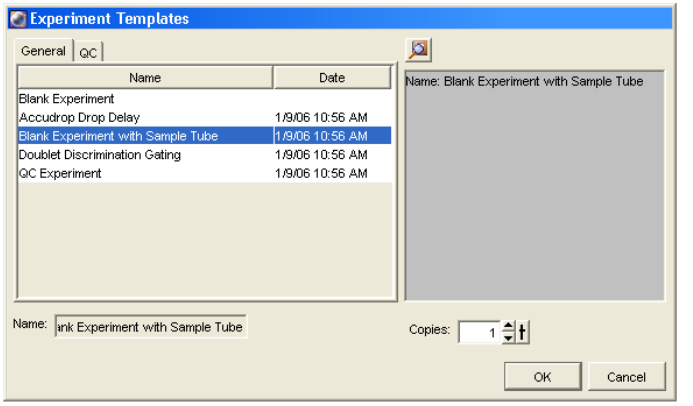
Templates

When you install BD FACSDiva software for the BD FACSAria instrument, the following additional experiment templates are installed in your BD Export\Templates directory:

- The Accudrop Drop Delay template contains a single specimen and tube, a gated plot on a standard worksheet, and a predefined sort layout. This experiment is used for setting the drop delay during sorting as described in Determining the Drop Delay on page 154.

- The Doublet Discrimination Gating template contains custom instrument settings, a single specimen and tube, and three gated plots on a global worksheet. This experiment is used to eliminate doublets as described in Optimization of Instrument Settings on page 126.

- The QC Experiment template contains instrument settings, a single specimen, three tubes, and three global worksheets containing gated plots and statistics for data from each laser. This experiment is used for daily instrument quality control, as described in Instrument Quality Control on page 113.



4

Running Samples

The following topics are covered in this chapter:

- Instrument Startup on page 104
- Instrument Quality Control on page 113
- Optimization of Instrument Settings on page 126
- Data Recording and Analysis on page 139
- Sample QC Log on page 147

Instrument Startup

Follow these steps to start up your BD FACSAria cell sorter.

1 Start up the workstation.

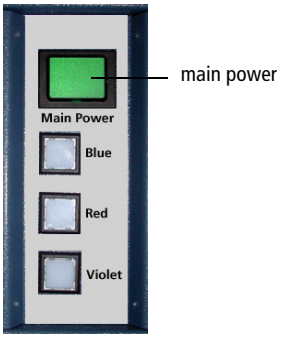
2 Open the flow cell access door.

The door is equipped with a laser interlock that blocks laser light when the door is open. Leave the flow cell access door open until you turn on the stream.

⚠ Do not allow the lasers to contact the cuvette flow cell before you activate the stream. Running the lasers without activating the stream can degrade the performance of the cuvette flow cell.

3 Turn on the instrument main power.

Figure 4-1 Power panel



If the instrument was just shut down, wait until the system is fully depressurized (stops hissing) before you turn on the main power.

4 Turn on the lasers; wait 30 minutes for them to warm up.

To turn on a laser, press the corresponding laser power button in the power panel (Figure 4-1).

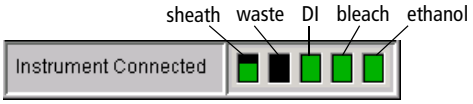
☑ **Tip** If you are using temperature control, start heating or cooling the sample injection chamber or the recirculating water bath while the lasers are warming up. See Sample Temperature on page 83.

5 Launch BD FACSDiva software, and log in with your user name and password.

To launch the software, double-click the application shortcut on the desktop.

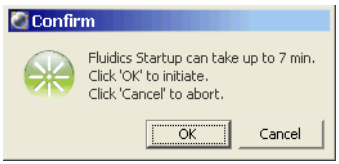
6 Verify fluidics levels in the Instrument window; replenish fluids or empty the waste, if needed.

To display the Instrument window, click the Instrument button in the Workspace toolbar ( ). Verify fluid levels at the bottom of the window.



To service the fluid containers, see Setting Up the Fluidics Cart on page 108 and Emptying the Waste on page 110.

7 From the BD FACSDiva Instrument menu, choose Fluidics Startup, and click OK at the message.



Fluidics startup removes DI water from the plenum tanks and fluid lines and replaces it with sheath fluid.

8 When fluidics startup is complete, use software controls to start the stream.

- Click the Sorting button in the Workspace toolbar ( ) to display the Breakoff and Side Stream windows.

- Click the Stream button in the Breakoff window ( ) to turn on the stream.

9 Open the sort block access door and check the stream position in the waste aspirator.

The stream should flow smoothly from the nozzle into the center of the waste aspirator.

If the stream is flowing but is unsteady, check for bubbles in the flow cell. If you see bubbles, turn off the stream, wait for the bubbles to float to the top, and turn on the stream again.

If you see any dripping or spraying or the stream image appears abnormal, turn off the stream and go to Troubleshooting the Stream on page 212.

10 Close the sort block access door.

11 Choose Sort > Sort Setup and verify the setup mode or choose a new mode.

For information about Sort Setup modes, see Sort Setup on page 92.

Setting Up the Breakoff

1 Adjust the Ampl. slider control until the drop breakoff is approximately in the center of the Breakoff window.

Do not exceed 70 volts. If you cannot achieve a drop breakoff at <70 volts, do the following:

- Check the flow cell for air bubbles. If you see bubbles, turn the stream off and back on.
- Make sure the sheath pressure and drop drive frequency are appropriate.

☑ **Tip** If the amplitude is <10 volts, turn on Attenuation in the Side Stream window.

2 Verify that the small satellite droplets are merging with the large droplets.

Satellite-merging is largely dependent on nozzle position. If the satellites are not merging, you might need to remove and reinstall the nozzle, making sure that the nozzle is pushed fully forward before turning the locking lever.

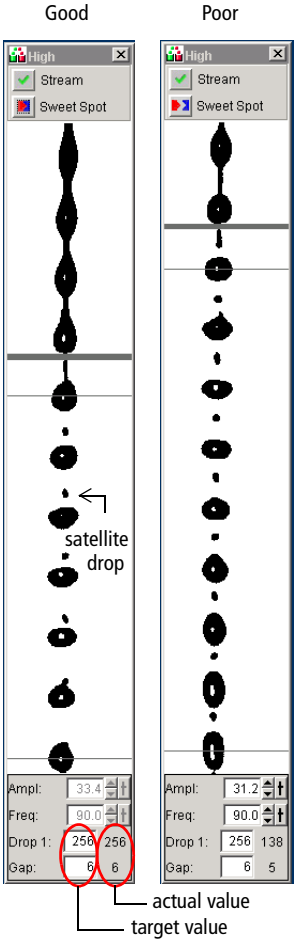
The satellite drops should merge into the drops in 6 satellites or less.

3 Input the actual Drop 1 value as the target in the Drop 1 field.

The actual value appears in the gray background next to the Drop 1 field. Once a valid target has been established, you do not usually need to reset it unless you change the nozzle or your Sort Setup option.

☑ **Tip** If the target value causes the Ampl. to exceed 70 volts, perform the recommendations under step 1 and re-input the Drop 1 target value.

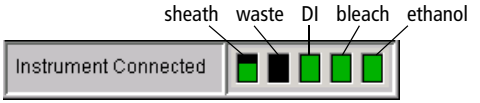
4 Turn on the Sweet Spot when the drop pattern is stable.



The Sweet Spot is designed to automatically adjust the drop drive amplitude to maintain the stability of the breakoff point. When the Sweet Spot is on, the Amplitude and Frequency fields are disabled; the values are automatically adjusted by the software. For more information, see Breakoff Window on page 64.

Setting Up the Fluidics Cart

☑ **Tip** Check the fluid levels in the sheath and waste containers every time you use the instrument. This ensures that you will not run out of sheath fluid during an experiment and that the waste container will not overflow. Fluidics level indicators are shown in the Instrument window in BD FACSDiva software.



If a fluidics container is running low, refill it with the fluid indicated on the container label. For best results, fill the containers only with the fluids shown in Table 4-1. For ordering information, see Reagents on page 239.

For instructions on emptying the waste, see page 110.

Table 4-1 Recommended fluids

Container	Compatible Fluid	Capacity
Sheath	• BD FACSFlow solution (non-sterile) or • 1X phosphate-buffered saline (PBS), with or without preservatives	Two 10-L containers or one 20-L cubitainer
Bleach	• 10% household bleach (0.5% sodium hypochlorite) or • BD™ FACSClean solution	5 L
DI	Deionized water	5 L
Ethanol	70% solution diluted in DI or laboratory-grade water	5 L

Refilling Containers

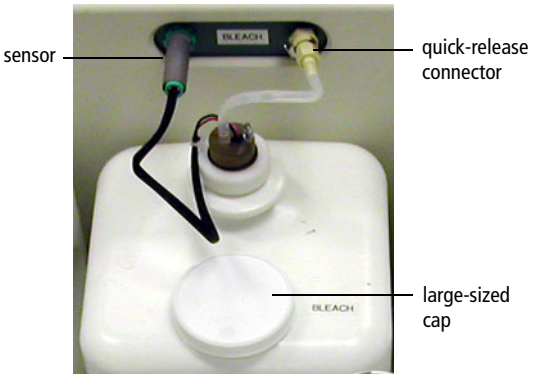
You can refill a container directly on the fluidics cart without detaching any lines, or you can remove the container for refilling. Note that during operation, you can add fluid to a container through the large cap without any interruption to your experiment, but if you detach any lines, you will need to prime the system.

If the instrument is run until the sheath supply is depleted, air will accumulate in the bubble filter. Purge the filter as described in Purging the Bubble Filter on page 208. If you are unable to remove all of the air, replace the bubble filter as described in Changing the Bubble Filter on page 197.

1 (Optional) Disconnect the container's sensor and quick-release connector if you need to move the container (Figure 4-2).

If you do not need to move the container, skip to step 2.

Figure 4-2 Fluidics container lines



2 Remove the large-sized cap from the container (Figure 4-2).

3 Fill the container with the fluid indicated on the container label.

4 Replace the container cap and hand-tighten it until it is fully closed.

5 Reconnect the sensor and quick-release connector to their respective ports, if needed.

Connect the lines if they were disconnected in step 1; see Figure 4-2 on page 109.

⚠ To ensure that the appropriate solutions are dispensed, do not switch tank positions. Make sure the label on each container matches the labelled port on the fluidics cart.

6 Prime the fluidics system if you disconnected the fluid lines in step 1.

From the BD FACSDiva main menu, choose Instrument > Cleaning Modes > Prime After Tank Refill.

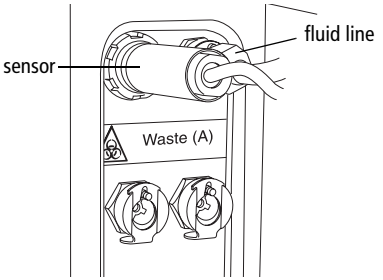
Emptying the Waste

Empty the waste tank daily and when the fluid indicator shows the waste is getting full. To prolong the life of the tank, BD Biosciences recommends that you switch to the alternate tank each time the waste is emptied.

⚠☣ All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, expose waste container contents to bleach (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

⚠☣ Change the waste tank cap every month to prevent tank pressurization. To order new replacement caps (BD part no. 33885407, pack of 12), contact your local BD Biosciences representative.

1 Disconnect the waste container's sensor and fluid line connectors from their respective ports on the fluidics cart.

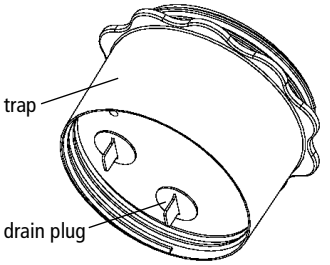


The waste tank can become pressurized when the cytometer is running. Always disconnect the tank from the wet cart before you empty it. Wait at least 1 minute for pressure to dissipate before you open the container.

2 Remove the disposable waste cap (large-sized cap) and attached trap from the container; place the assembly on the bench label-side up.

Do not wet the cap on top of the trap. If you see liquid inside the trap, remove the drain plug and fully drain the liquid before you replace the plug (Figure 4-3 on page 112).

Figure 4-3 Draining liquid from the trap



3 Empty the waste tank according to your standard laboratory procedures for biohazardous waste.

4 Add approximately 1 L of bleach to the waste container (10-L container).

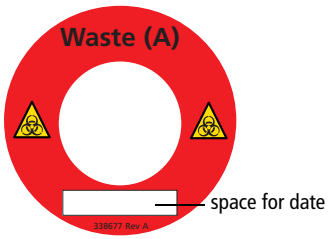
Add a sufficient amount so that 10% of the total waste volume is bleach.

5 Replace the waste trap and attached filter cap. Hand-tighten the trap and cap until they are fully closed.

To prevent overpressurization during fluidics startup, do not overtighten the trap or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants such as Teflon tape or other adhesives.

6 If one month has passed since you last changed the cap, replace the filter cap with a new one.

Tip When you replace the cap, write the date on the new cap as a reminder.



7 Reconnect the sensor and fluid line connectors to their respective ports.

Instrument Quality Control

Use instrument quality control (QC) to ensure consistent instrument performance over time.

During instrument QC, you want to keep as many parameters constant as possible. For example, always use the same QC particle type and lot number, sheath pressure, and PMT settings. If you work with different sheath pressures, you might consider having a separate QC Experiment for each pressure.

When the experiment, instrument settings, and QC sample are constant, changes in the recorded means and CVs indicate variations in instrument performance over time. QC data should be analyzed for trends over 30–60 runs.

This section describes how to use a QC experiment template to verify laser delay and area scaling values, and how to record parameter means and CVs for a fluorescent bead. For examples of fluorescent particles that can be used for instrument QC, see Instrument Setup Particles on page 238.

NOTICE QC results are affected by laser and fluidics performance. BD strongly recommends following the laser and fluidics maintenance procedures in Chapter 6.

Setting Up the QC Experiment

The steps in this section show you how to set up an experiment for instrument QC. If you have already created a similar experiment, you can reuse it by duplicating the most recent specimen. See Reusing the Experiment on page 125.

1 Choose Instrument > Instrument Configuration and set the current configuration to *FACSAria*.

☑ **Tip** The FACSAria configuration contains the most complete set of fluorochromes, thus should be applicable for most assays.

2 Make sure the octagon and trigon(s) contain appropriate filters.

For assistance, see Application Options on page 242.

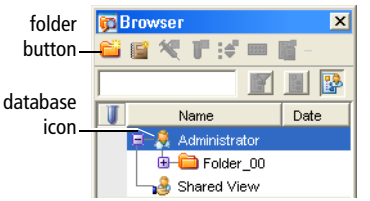
⚠ For accurate data results, the instrument optics must match the current instrument configuration.

3 Click the corresponding buttons in the Workspace toolbar to display the Browser ( ), Instrument ( ), Inspector (, Worksheet ( ), and Acquisition Dashboard ( ) windows, as needed.

☑ **Tip** As you work in the software, windows can become hidden. You can easily bring a window to the forefront by double-clicking the corresponding button in the Workspace toolbar.

4 (Optional) Create a folder for instrument QC.

In the Browser, select the icon for your database and click the New Folder button in the Browser toolbar.



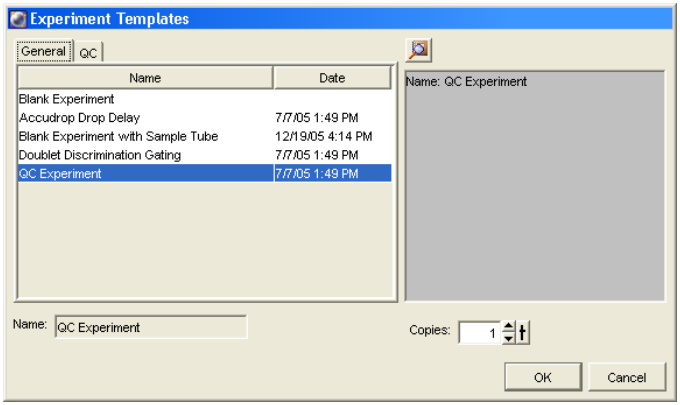
Rename the folder with your name. Alternatively, name the folder *Instrument QC* or create an instrument QC folder inside another folder. Refer to the *BD FACSDiva Software Reference Manual* for ideas on how to organize experiments.

☑ **Tip** To place an experiment inside a folder, select the folder before you create the experiment.

5 Create an experiment from the QC Experiment template.

Choose Experiment > New Experiment, click the General tab, and select the QC Experiment template (Figure 4-4 on page 115). Click OK to add a copy of the template to your Browser.

Figure 4-4 Creating an experiment from the QC template

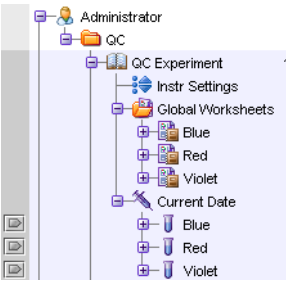


6 Add the current month and year to the experiment name; rename the specimen with today's date.

7 Expand the Global Worksheets folder and the dated specimen in the Browser.

The QC experiment template contains three global worksheets and three tubes, each intended for verifying signal from one laser. For instance, the Blue tube and Blue global worksheet will be used to verify signal for 488-nm laser parameters.

Your Browser should look similar to the following:



Running QC Particles

In addition to its Browser elements and analysis objects, the QC experiment template includes instrument settings from when the experiment template was created. The following sections describe how to fine-tune the settings by placing the signals for each parameter at channel 100,000. The data shown is from SPHERO™ Rainbow Calibration Particles.

Optimizing Signal from the Blue Laser

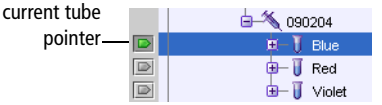
1 Prepare the beads according to the manufacturer's instructions.

2 Install the tube of fluorescent beads onto the loading port.

☑ **Tip** Ensure that the appropriate tube holder is installed.

3 Click the tab to display the *Blue* global worksheet.

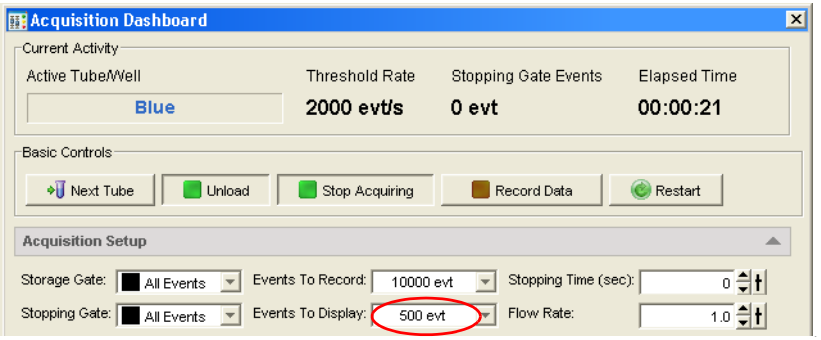
4 Click to set the current tube pointer next to the *Blue* tube in the Browser.



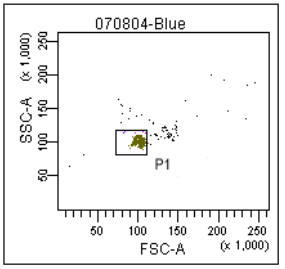
5 Click Load in the Acquisition Dashboard.

The loading port rises to enclose the tube within the chamber. Once the tube is loaded, acquisition starts automatically.

6 Set the Events to Display to 500 events.



7 Adjust the FSC gain and the SSC voltage to place the particles within the *Singlet* gate at approximately 100 x 10^3 in the FSC vs SSC dot plot:



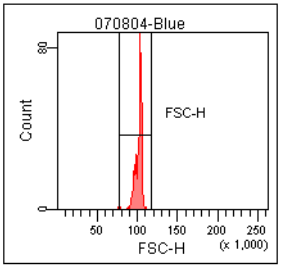
- Select the *Blue* tube in the Browser.
- Click the Parameters tab in the Instrument window.
- Click the up and down arrows or drag the sliders to adjust the values.

Verifying Area Scaling

BD FACSDiva software uses area as its default parameter. Fluorescence area measurements can be affected by sheath pressure and particle size. FSC area signal can differ because the signal is detected by a photodiode, unlike the other parameters which use photomultiplier tubes to detect the signal. For these reasons, it is important to verify area scaling for each experiment performed on the BD FACSAria instrument.

Refer to the *BD FACSDiva Software Reference Manual* for more information about area scaling.

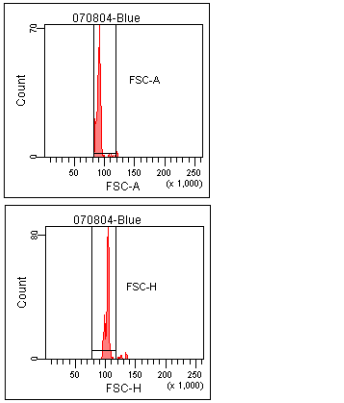
1 Adjust the FSC voltage to place the FSC-H signal at approximately 100 x 10^3:

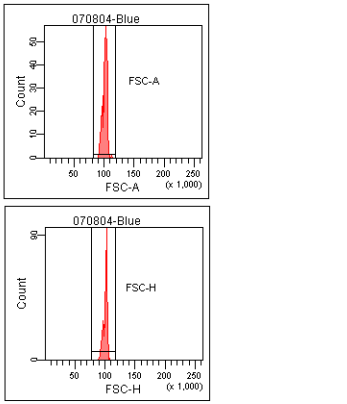


2 Adjust the Singlet gate to encompass the singlet population, if necessary.

3 Compare the signal intensity of FSC-A and FSC-H (Figure 4-5).

Figure 4-5 FSC area scaling before (left) and after (right) adjustment





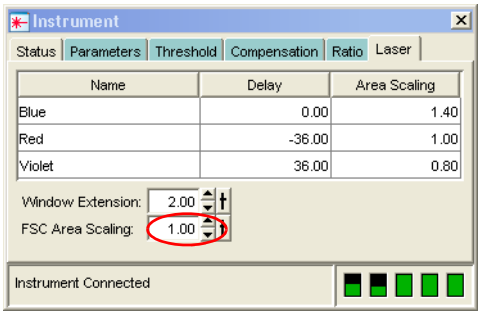
Experiment Name: QC July 04
Specimen Name: 070804
Tube Name: Blue
Record Date:
$OP:

Population	FSC-A Mean	FSC-A CV	FSC-H Mean	FITC-A Mean	FITC-A CV	FITC-H Mean
FSC-A	89,391	5.0	101,997	100,686	5.6	101,717
FSC-H	88,898	3.4	101,467	100,035	2.8	101,127
FITC-A	88,898	3.4	101,467	100,035	2.8	101,127
FITC-H	88,898	3.4	101,467	100,035	2.8	101,127

Experiment Name: QC July 04
Specimen Name: 070804
Tube Name: Blue
Record Date:
$OP:

Population	FSC-A Mean	FSC-A CV	FSC-H Mean	FITC-A Mean	FITC-A CV	FITC-H Mean
FSC-A	99,931	3.7	99,145	100,216	6.4	101,399
FSC-H	99,931	3.7	99,145	100,216	6.4	101,399
FITC-A	99,902	3.7	99,134	99,820	2.9	101,048
FITC-H	99,902	3.7	99,134	99,820	2.9	101,048

If they are the same, there is no need to adjust area scaling. If they are not, adjust FSC area scaling in the Laser tab of the Instrument window:

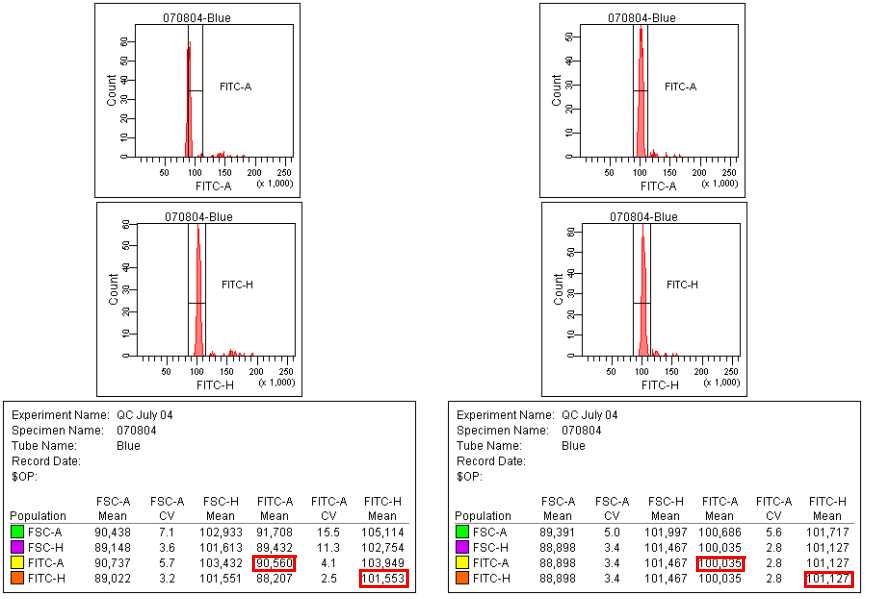


- If FSC-A signal is lower than FSC-H, increase FSC area scaling.
- If FSC-A signal is higher than FSC-H, decrease FSC area scaling.
- Move the interval gates as needed to encompass peaks.
- Continue adjusting the scaling until the area signal is similar to the height.

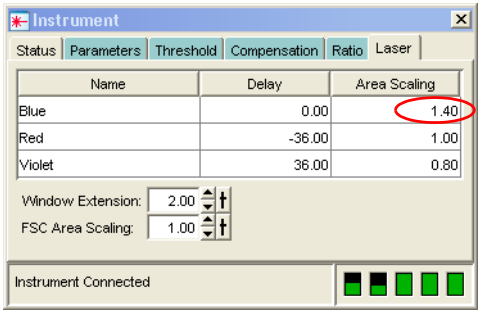
4 Adjust the FITC voltage to place the FITC-H signal at approximately 100×10^3.

5 Compare the signal intensity of FITC-A and FITC-H (Figure 4-6 on page 120).

Figure 4-6 Blue laser area scaling before (left) and after (right) adjustment



If they are the same, there is no need to adjust area scaling. If they are not, adjust area scaling for the Blue laser in the Laser tab:



- If FITC-A signal is lower than FITC-H, increase area scaling.
- If FITC-A signal is higher than FITC-H, decrease area scaling.

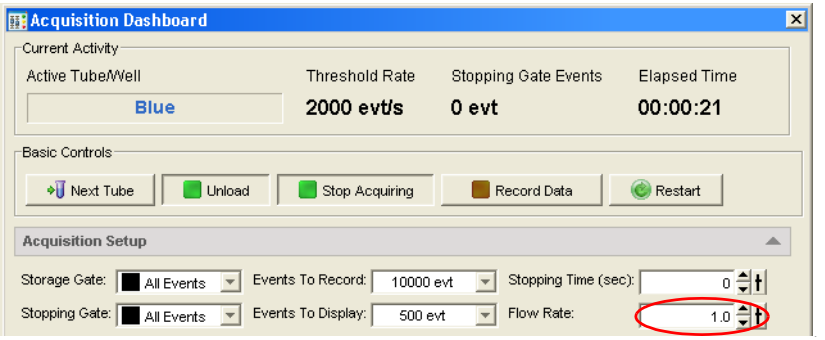
- Move gates as needed to encompass the singlet population and the FITC-A and FITC-H populations.

- Continue adjusting the scaling until the area signal is similar to the height.

6 Adjust the PMT voltage for each parameter to set the signal at approximately 100 x 10^3.

Recording Data

1 Set the flow rate to 1.0.



2 Click Record Data.

After data has been recorded, a disk is added to the tube icon, and a copy of the experiment's instrument settings is saved with the tube.

3 Click Unload in the Acquisition Dashboard and remove the tube.

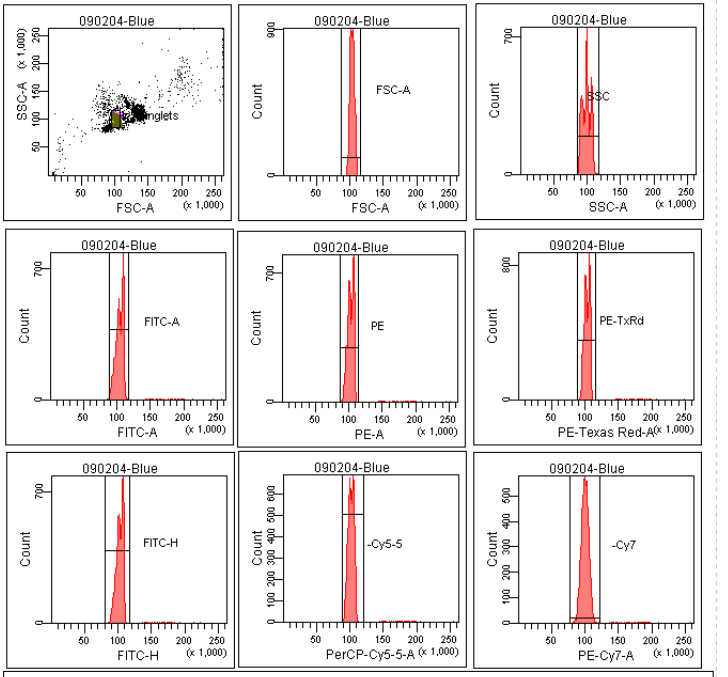
4 Adjust interval gates to encompass peaks, if needed.

5 (Optional) Print the *Blue* worksheet with the *Blue* tube data displayed.

- Choose File > Print.
- Select pages 1 to 1 in the Print Range section of the Print dialog.

An example of recorded data is shown in Figure 4-7.

Figure 4-7 Blue laser data (example)



Experiment Name: QC Sept 03 jg
Specimen Name: 090204
Tube Name: Blue
Record Date: Sep 2, 2004 7:45:14 AM
$OP: Jena

Population	SSC-A Mean	SSC-A CV	PE-A Mean	PE-A CV	PE-Texa... Mean	PE-Texa... CV	PerCP-... Mean	PerCP-... CV	PE-Cy7-A Mean	PE-Cy7-A CV
singlets	97,620	6.1	101,704	7.8	102,034	7.5	100,749	7.8	99,225	8.6
SSC	97,620	6.1	101,704	7.8	102,034	7.5	100,749	7.8	99,225	8.6
PE	97,560	6.1	101,170	4.3	101,503	3.9	100,208	4.1	98,683	5.3
PE-TxRd	97,560	6.1	101,170	4.3	101,503	3.9	100,208	4.1	98,683	5.3
-Cy5-5	97,560	6.1	101,170	4.3	101,503	3.9	100,208	4.1	98,683	5.3
-Cy7	97,560	6.1	101,170	4.3	101,503	3.9	100,208	4.1	98,683	5.3

(data from page 1)

Optimizing Signal from the Red Laser

In addition to area scaling and PMT voltages, you will need to adjust the laser delay setting to optimize signal from the red laser.

1. Click the tab to display the *Red* global worksheet.

2. Move the current tube pointer to the *Red* tube.

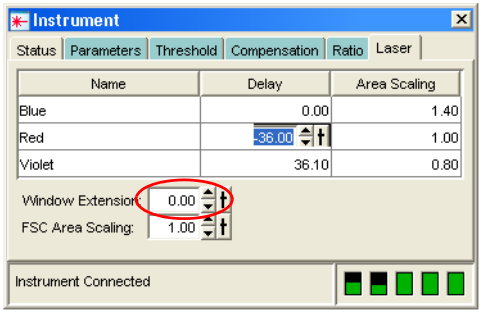
3. Install the tube containing fluorescent particles for the red laser onto the loading port, and click Load in the Acquisition Dashboard.

4. Adjust the APC-Cy7 voltage to place APC-Cy7-H signal at approximately 100 x 10^3.

5. Adjust the delay setting for the red laser, as needed.

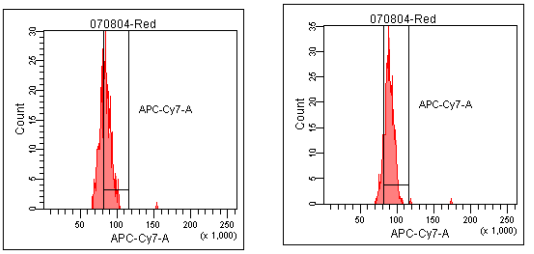
   The sort setup inputs area scaling and laser delay values from the last time the sort setup was used. If adjustments are needed, they should be slight. Verify the delay setting in the APC-Cy7 plot while making adjustments in the Laser tab: the delay setting is optimized when fluorescent signal intensity is at its highest.

   - Change the window extension to zero.



   - Adjust the delay for the Red laser until APC-Cy7-A signal is at its highest (Figure 4-8 on page 124).

Figure 4-8 Laser delay before (left) and after (right) adjustment



6 Re-input the previous window extension setting (typically 2).

7 Compare the signal intensity of APC-Cy7-A and APC-Cy7-H.

If they are the same, there is no need to adjust area scaling. If they are not, adjust area scaling for the Red laser in the Laser tab:

- If APC-Cy7-A signal is lower than APC-Cy7-H, increase area scaling.
- If APC-Cy7-A signal is higher than APC-Cy7-H, decrease area scaling.
- Move gates as needed to encompass the singlet population and the APC-Cy7-A and APC-Cy7-H populations.
- Continue adjusting the scaling until the area signal is similar to height.

8 Adjust the PMT voltage for the APC parameter to set the signal at approximately 100 x 10^3 in the corresponding histogram.

Recording Data

1 Ensure the current tube pointer is next to the *Red* tube, and click Record Data.

2 After data has been recorded, click Unload and remove the tube.

3 Adjust interval gates to encompass peaks, if needed.

4 (Optional) Print the *Red* worksheet with the *Red* tube data displayed.

Optimizing the Signal from the Violet Laser

Repeat the steps in Optimizing Signal from the Red Laser on page 123, using the *Violet* worksheet and *Violet* tube.

Tracking QC Results

To track instrument performance over time, copy PMT voltages, means, and CVs for each fluorescence parameter onto a QC log such as the one provided on page 147. Alternatively, print the experiment's instrument settings or Instrument Status report and a copy of each worksheet and keep a copy of the printouts.

The instrument settings printout contains PMT voltages and compensation settings; the Instrument Status report includes the sheath pressure and flow rate, lasers used, laser delay, area scaling, and window extension settings in addition to PMT voltages and compensation settings.

- To print instrument settings, right-click the Instr Settings icon for the experiment and choose Print, or select the icon and click Print in the Inspector.

- To print the report, set the current tube pointer on the last-used tube and choose Instrument > Instrument Status Report. Choose File > Print Report in the report window.

Reusing the Experiment

To reuse this experiment for QC on another day, do the following.

1. Open the instrument QC experiment.

2. Right-click the most recent specimen and choose Duplicate without Data.

   The three QC tubes appear under the new specimen.

3. Rename the specimen with the current date.

4 Click the tab to display the *Blue* worksheet; move the current tube pointer to the *Blue* tube.

5 Continue with the QC procedure.

- For the blue laser, verify area scaling.
- For the red and violet lasers, verify laser delay and area scaling settings.

Optimization of Instrument Settings

Before you record data for a sample, instrument settings should be optimized to position the cells of interest on scale for scatter and fluorescence parameters.

In general, the following settings are optimized for each sample type and fluorochrome used:

- FSC and SSC voltages
- FSC threshold
- FSC and fluorescence area scaling
- fluorescence PMT voltages
- compensation

Each adjustment is explained in detail in the sections that follow. It is important that you adjust these settings in order, as some adjustments influence others. You might need to vary certain steps for different sample types.

To demonstrate these adjustments, the following sections describe how to optimize settings for four-color BD Calibrite™ beads. Compensation will be automatically calculated using the Instrument Setup feature. For more information about this feature, refer to the *BD FACSDiva Software Reference Manual*. If you are performing compensation manually, not all steps will apply.

Creating the Experiment

Before you begin optimizing settings, it is important to verify the instrument configuration and create an experiment containing appropriate parameters for the assay.

1 Choose Instrument > Instrument Configuration, and verify the current configuration.

☑ **Tip** The FACSAria configuration contains the most complete set of fluorochromes, thus should be applicable for most assays.

2 Make sure the octagon and trigon(s) contain appropriate filters.

For assistance, see Application Options on page 242.

⚠ For accurate data results, the instrument optics must match the current instrument configuration.

3 Click the corresponding buttons in the Workspace toolbar to display the Browser ( ), Instrument ( ), Inspector ( ), Worksheet ( ), and Acquisition Dashboard ( ) windows, as needed.

4 (Optional) Create a folder for your experiment.

Select the icon for your database and press Ctrl-N; rename the folder appropriately. Refer to the *BD FACSDiva Software Reference Manual* for ideas on how to organize experiments.

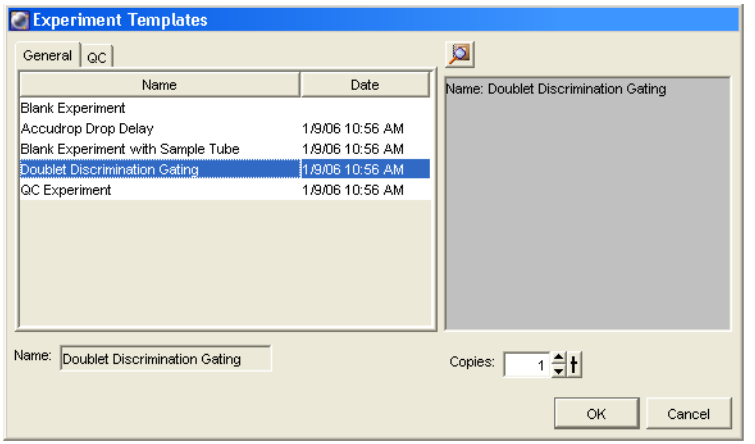
☑ **Tip** To place an experiment inside a folder, select the folder before you create the experiment.

5 Create an experiment from the Doublet Discrimination Gating template.

For best results, BD Biosciences recommends that you perform doublet discrimination in order to record only singlet events. This template contains instrument settings and gated plots for this purpose; a procedure is given in Recording Data on page 142.

For experiments where you do not need to distinguish singlets from doublets, you can start with a default experiment or a template of your choice.

To use the template, choose Experiment > New Experiment and select the Doublet Discrimination Gating template. Click OK to add a copy of the template to your Browser.



6 Rename the experiment appropriately, such as *4-Color Calibrite*.

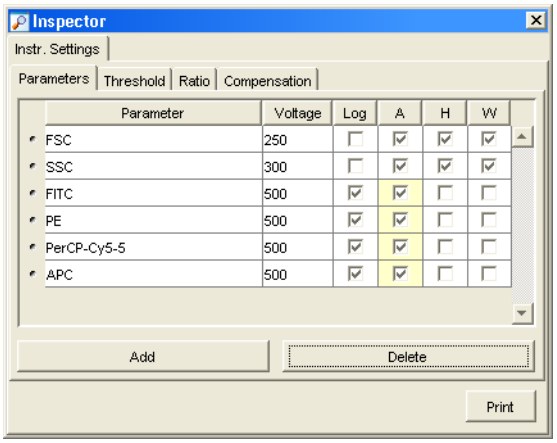
7 Select the experiment-level instrument settings in the Browser; click on the Parameters tab and delete any unneeded parameters (Figure 4-9 on page 129).

For this example, delete all parameters except FSC, SSC, FITC, PE, PerCP-Cy5.5, and APC.

To delete parameters, press the Ctrl key and click the selection button next to each parameter that is not used in the experiment. When all rows have been selected, press the Delete key or click the Delete button in the Inspector.

To change to a different parameter than what is listed, click the parameter name and choose a different fluorophore from the drop-down menu.

Figure 4-9 Parameters for four-color BD Calibrite beads

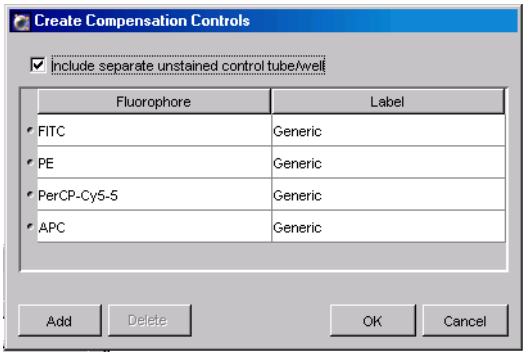


8 Choose Instrument > Instrument Setup > Create Compensation Controls.

The Create Compensation Controls dialog box appears, listing only those parameters specified in the previous step.

9 Click OK to add the specified controls.

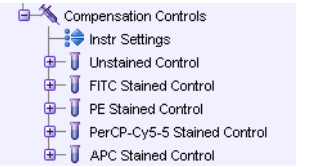
Alternatively, add and define label-specific controls, and then click OK.



Add label-specific controls when your experiment contains samples stained with the same fluorophore conjugated to different antibodies (labels) that require different compensation values. This is especially noticeable in

tandem conjugates due to lot-to-lot variation. Refer to the *BD FACSDiva Software Reference Manual* for more information about this feature.

A compensation specimen is added to the experiment, along with a stained control tube for each compensation control. (Expand the specimen to view all tubes.) Worksheets containing appropriate plots are added for each compensation tube.

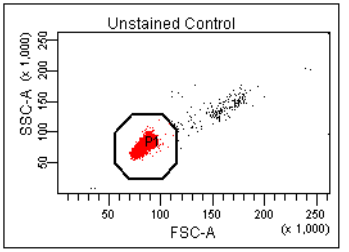


Adjusting the Voltages and Threshold

The unstained control will be used to adjust the FSC, SSC, and FSC threshold and to gate the population of interest (bead singlets, in this case).

1. Install the unstained control tube on the cytometer.
2. Expand the compensation specimen in the Browser.
3. Click to set the current tube pointer next to the unstained control tube and click Load.
4. Adjust the FSC and SSC voltages to appropriately display the scatter properties of the unstained control (Figure 4-10).

Figure 4-10 Voltages adjusted



5. Click the Threshold tab and adjust the FSC threshold, if needed.

   Set the threshold to remove most of the debris without cutting off the singlet population (Figure 4-10).

6 Move the P1 gate to surround only the singlets (Figure 4-10).

Select the gate by clicking on the boundary and drag to move it.

To change its shape, right-click the boundary and choose Recalculate.

7 Right-click the P1 gate and choose Apply to All Compensation Controls.

The P1 gate on each Stained Control worksheet is updated with your changes.

8 (Optional) Click Stop Acquiring to stop acquisition of the unstained control.

This allows you to save sample while you are setting up for the next adjustment.

Adjusting Area Scaling

The required amount of area scaling depends on your sheath pressure, particle size, and concern for not oversaturating the PMTs. FSC and laser-specific area scaling should be verified each time the sheath pressure or particle size changes.

FSC Area Scaling

1 Ensure the FSC-H checkbox is selected in the Parameters tab.

If you are using the Doublet Discrimination Gating template, the checkbox is already selected.

2 Create two histogram plots on the Unstained Control worksheet.

3 Change one histogram plot axis to FSC-H.

4 If acquisition is stopped, start acquisition of the unstained control tube.

Click the current tube pointer next to the unstained control tube.

5 Adjust FSC area scaling in the Laser tab until FSC-A signal is similar to FSC-H.

   Adjust the scaling until the histogram peak is at approximately the same value in the two histograms.

6 Readjust the FSC and SSC voltage or P1 gate, if needed.

   If you adjust the P1 gate, right-click on the boundary and choose Apply to All Compensation Controls.

7 Unload the unstained control tube.

Primary Laser Area Scaling

This section describes how to adjust area scaling for the 488-nm (Blue) laser.

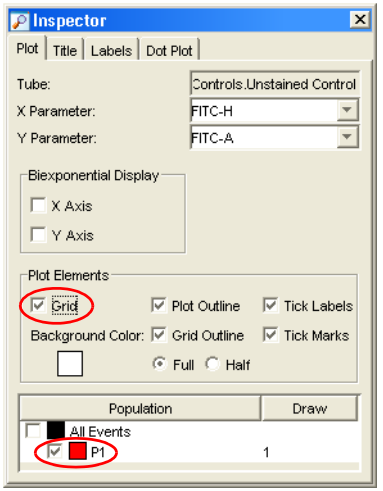
1 Select the FITC-H checkbox in the Parameters tab.

   When you choose height for one fluorescent parameter, the height checkboxes for all other fluorescent parameters are selected as well.

2 Create a dot plot on the Unstained Control worksheet.

3 Change the plot axes to FITC-H vs FITC-A.

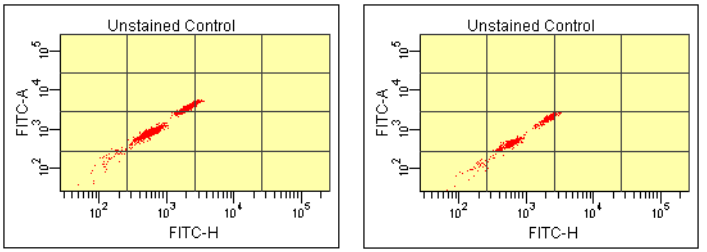
4 In the Plot Inspector, select the Grid and P1 population checkboxes.



5 Install the FITC stained control on the loading port and click Load.

6 Adjust area scaling for the Blue laser until the signal fits the plot diagonally.

Figure 4-11 Area scaling unadjusted (left) and adjusted (right)



- If the area is greater than the height, decrease area scaling.
- If the area is less than the height, increase area scaling.

7 Check the FSC-A vs SSC-A plot and readjust the FSC and SSC voltage or P1 gate, if needed.

   Area scaling affects the signal for all parameters from the blue laser, thus you might need to readjust the scatter parameters.

   If you adjust the P1 gate, right-click on the boundary and choose Apply to All Compensation Controls.

8 Continue adjusting area scaling until the signal fits the plot diagonally.

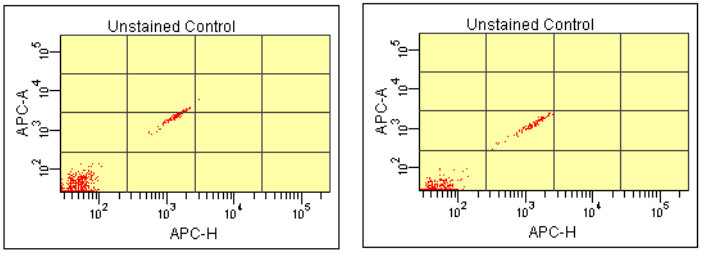
9 Unload the FITC stained control.

Second Laser Area Scaling

This section describes how to adjust area scaling for the 633-nm (Red) laser. For the violet laser, substitute appropriate parameters.

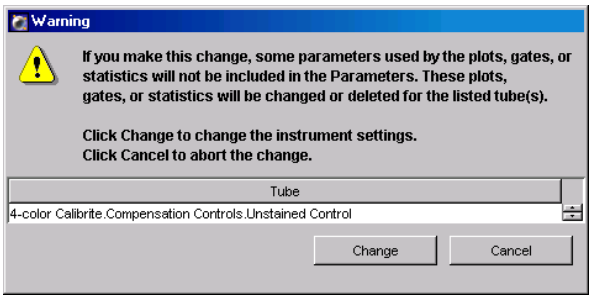
1. Change axes of the fluorescence dot plot to APC-H vs APC-A.
2. Install the APC stained control on the loading port and click Load.
3. Adjust area scaling for the Red laser until the signal fits the plot diagonally.

Figure 4-12 Area scaling unadjusted (left) and adjusted (right)



- If the area is greater than the height, decrease area scaling.
- If the area is less than the height, increase area scaling.

4. Unload the APC stained control.
5. Deselect the height checkbox in the Parameters tab.
6. Click Change in the dialog box that appears:


Warning
If you make this change, some parameters used by the plots, gates, or statistics will not be included in the Parameters. These plots, gates, or statistics will be changed or deleted for the listed tube(s).
Click Change to change the instrument settings.
Click Cancel to abort the change.
Tube
4-color Calibrite Compensation Controls.Unstained Control
Change
Cancel

Optimizing Fluorescence PMT Voltages

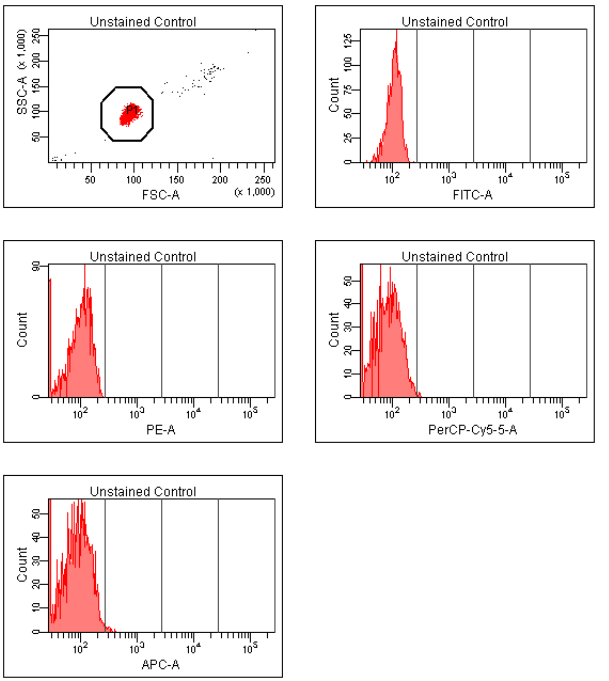
1 Display the log grid in all histogram plots.

- Press Ctrl-A to select all plots on the Unstained Control worksheet.
- Select the Grid checkbox in the Plot Inspector.

In a four-log display, values are displayed from 26–262,143. Thus, the first log decade ranges from 26–262. Gridlines are used to delineate log decades on plots, and are shown only on plots displaying log parameters.

2 Load the unstained control tube.

3 Optimize the voltages to place the negative population for each fluorescent parameter within the first log decade:



4 Click Record Data.

5 When recording is finished, click Unload and remove the unstained control tube from the cytometer.

⚠ Do not change the PMT voltages after the first compensation control has been recorded. In order to calculate compensation, all controls must be recorded with the same PMT voltage settings. If you need to adjust the PMT voltage for a subsequent compensation control, you will need to record all compensation controls again.

Calculating Compensation

Before you can calculate compensation, you will need to record data for each single-stained control.

1 Install the first stained control tube onto the cytometer.

2 In the Acquisition Dashboard, click Next Tube, and then Load.

Next Tube moves the current tube pointer to the next tube in the Browser. Alternatively, click to move the pointer to the next tube.

3 Click Record Data when events appear in the plots.

4 When recording is finished, click Unload and install the next stained control tube onto the cytometer.

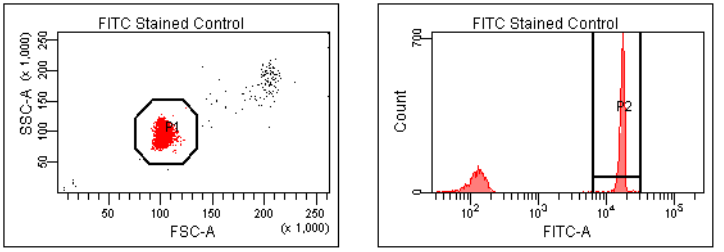
5 Repeat steps 2 through 4 until data for all stained control tubes has been recorded.

6 Double-click the first stained control tube to display the corresponding worksheet.

7 Verify that the Snap-to Interval gate encompasses the positive population (Figure 4-13 on page 138).

Adjust the gate, if needed.

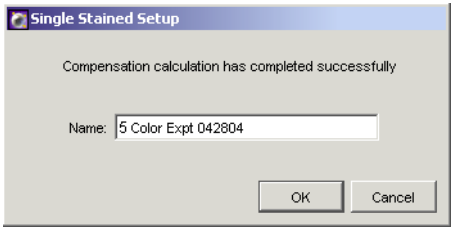
Figure 4-13 Gating the positive population



8 Repeat steps 6 and 7 for the remaining compensation tubes.

9 Choose Instrument > Instrument Setup > Calculate Compensation.

10 Enter the name of your experiment as the setup name, and click OK.



☑ **Tip** To keep track of compensation setups, include the experiment name, date, or both in the setup name.

NOTICE BD Biosciences recommends that you always visually and statistically inspect automatically calculated overlap values. The means of the positive controls should be aligned with the means of the negative.

Data Recording and Analysis

Once you have optimized the instrument electronics for your sample type, you are ready to record and analyze data.

Before you record data, BD Biosciences recommends that you gate out doublets in order to record only singlet events. The Doublet Discrimination Gating template provides gated plots for this purpose.

During analysis, recorded data is displayed in plots, and gates are used to define populations of interest. BD FACSDiva software analyzes the gated data and calculates statistics that you can print or export. With global worksheets, data can be shown for a series of tubes on the same worksheet, manually or in an automated batch analysis.

The following sections describe how to use BD FACSDiva software features to record and analyze sample data. To demonstrate, data will be recorded and analyzed for a four-color sorting experiment using BD Calibrite beads.

Setting Up the Experiment

Before you record data, set up an experiment with appropriate tubes, plots, and labels for your assay. This section describes how to add Browser and worksheet elements to the experiment that was started in the previous section, Optimization of Instrument Settings.

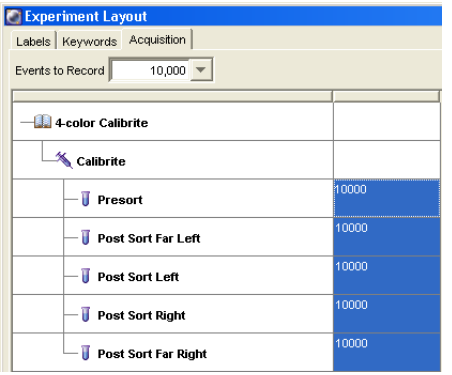
1. Collapse the Compensation Specimen to save room in the Browser.
2. Rename Specimen_001 as *Calibrite*.
3. Rename Tube_001 as *Presort*.
4. Create four additional tubes and rename them *Post Sort Far Left, Post Sort Left, Post Sort Right,* and *Post Sort Far Right*.

☑ **Tip** Press the Enter key twice to move to the next tube without using the mouse.

5 Use the Experiment Layout dialog box to specify the number of events to record for each tube.

The Experiment Layout can be used to define the events to record as well as parameter labels for all tubes in an experiment. Labels appear on the plot axes and in all statistics views.

- Choose Experiment > Experiment Layout.
- On the Acquisition tab, select the events to record field for all *Calibrite* specimen tubes, and choose or enter 10,000 events.



- Click OK. Notice that the Acq. tab in the Inspector updates automatically.

Setting Up the Global Worksheet

A global worksheet will be used to perform doublet discrimination, and to set up plots to preview and record pre- and post-sort data.

1 Click the Worksheets View button on the Worksheet toolbar ( ).

The global worksheet is shown. If you are using the Doublet Discrimination Gating template, the worksheet shows the predefined plots and gates intended to help classify singlets from doublets.

The second and third plots are set up to display gated data from the first and second plots, respectively, as shown in the population hierarchy view.

2 (Optional) Rename the global worksheet.

For example, rename it *Record Data* or *Doublet Discrimination*.

3 Create two dot plots for previewing and recording data.

For this example, create a FITC vs PE plot and an APC vs PerCP-Cy5.5 plot.

☑ **Tip** Double-click the Plot button to keep it selected until you create all plots. Click any other button to undo the selection.

4 Set up the fluorescence plots to display data from the FSC gate.

The FSC gate defines singlet events. By gating the fluorescence plots through this population, only singlet events will be recorded.

- Select the two plots.
- In the Inspector, select the FSC Gate checkbox.

5 Arrange the fluorescent plots so they fill the page vertically.

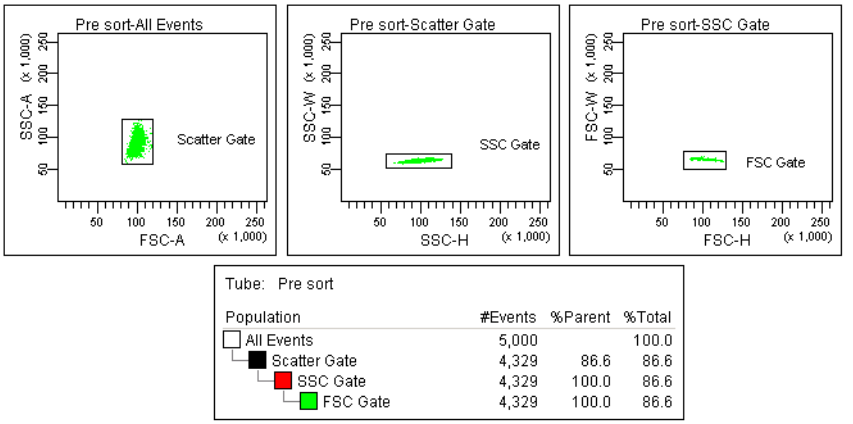
For an example, see Figure 4-15 on page 145.

Recording Data

This section describes how to adjust the gates to eliminate doublets and record singlet events.

1. Move the current tube pointer to the Presort Tube.
2. Install the mixed BD Calibrite tube onto the loading port and click Load.
3. Change the Events to Display to 5000 events.
4. Adjust the *Scatter Gate* to encompass the singlet events.
5. Adjust the *SSC Gate* to encompass the low SSC-W population.
6. Adjust the *FSC Gate* to encompass the low SSC-W population.

Figure 4-14 Discriminating singlet events



7. Alt-click the current tube pointer to record data.
8. When all events have been recorded, click Unload and remove the tube from the cytometer.
9. (Optional) Print the experiment-level instrument settings or the Instrument Status report.

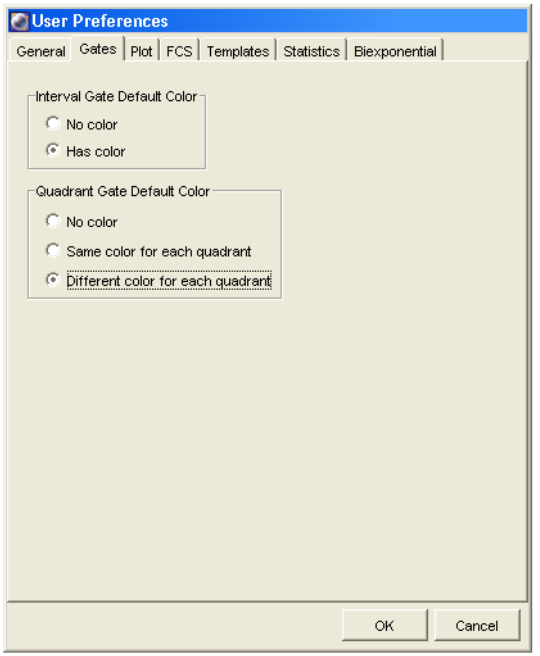
To print the settings, right-click the instrument settings icon and choose Print.

Analyzing Data

This section describes how to set up plots, gates, and a statistics view to analyze the recorded data. By the end of this section, your analysis should look similar to that shown in Figure 4-15 on page 145.

1 Choose Edit > User Preferences.

2 On the Gates tab, set the preferences as follows:



3 Create the following gates:

- Create a Quadrant gate on the FITC vs PE plot.
- Create an Interval gate to capture the APC beads.
- Create a Rectangle gate to capture the PerCP-Cy5.5 beads.

4 Rename each population in the Population Hierarchy view.

☑ **Tip** Press the Enter key twice to move to the next population without using the mouse.

5 Right-click either fluorescent plot and choose Create Statistics View.

A default statistics view is added to the worksheet.

6 Right-click the statistics view and choose Edit Statistics View.

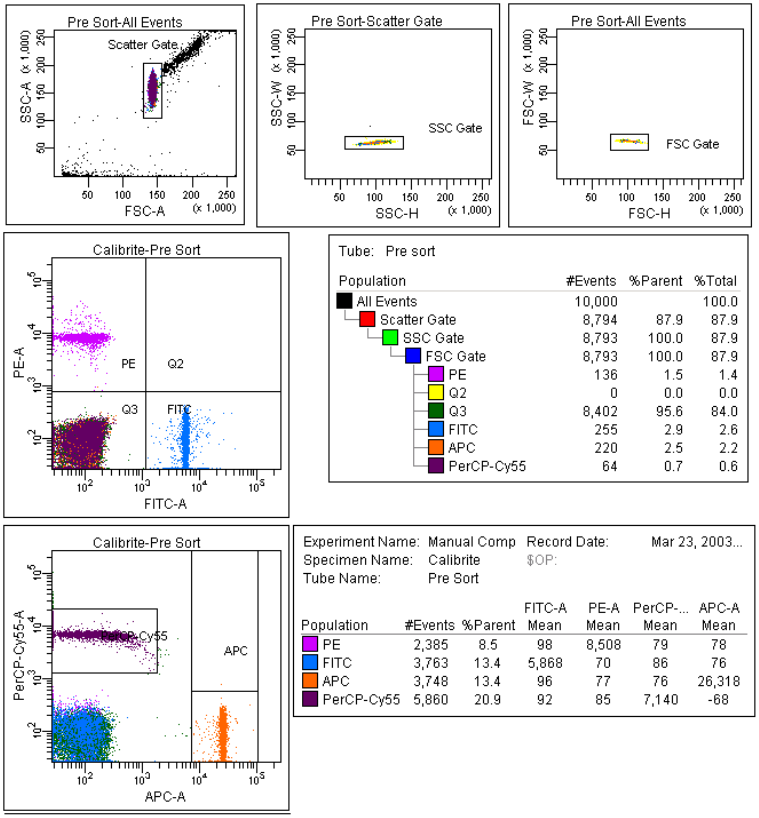
7 Edit the statistics view as follows:

- In the Header tab, select the checkbox to *Use 2 columns for display.*
- On the Populations tab, deselect the checkboxes for all populations except FITC, PE, PerCP-Cy5.5, and APC.
- On the Statistics tab, select the Mean checkboxes for the fluorescence-A parameters.

8 Resize the statistics view so it fits on the page.

9 (Optional) Print the analysis.

Figure 4-15 BD Calibrite analysis for mixed-bead tube



Saving the Analysis

Now that the analysis strategy has been defined, you can use it to analyze post-sort data for the remaining tubes in the experiment. Global worksheets allow you to apply an analysis strategy to a series of data files, either during recording or for a series of recorded tubes.

☑ **Tip** Use batch analysis to automatically process a series of data files from recorded tubes. You can print worksheets or export statistics using options in the batch analysis dialog box.

When data is analyzed on a global worksheet, the analysis will not be saved with each tube. Do the following to automatically save a copy of the analysis with each tube as it is recorded.

1. Choose Edit > User Preferences, and enable the preference to *Save analysis after recording...*

2. (Optional) To place each tube's analysis on a new worksheet, also enable the *Tube-specific worksheet* preference.

   This preference automatically starts a new worksheet for each tube's analysis. Refer to the *BD FACSDiva Software Reference Manual* for more information about user preferences.

3. Click the Worksheets View button ( ) to switch to the standard worksheet view (gray-tinted tabs).

4. Click the tab to display Sheet1.

   When a tube is recorded, the analysis is copied to the top-most worksheet displayed. After compensation calculation, a compensation worksheet is usually the top-most worksheet. Switch to Sheet1 to copy the analysis to a blank worksheet.

   To locate the worksheet, click the left arrow control or press the arrow key on your keyboard until the worksheet is displayed. Alternatively, choose Worksheet > New Worksheet to create a new blank worksheet.

5 Click the Worksheets View button ( ) to return to the global worksheet view (green-tinted tabs).

6 Move the current tube pointer to the Post Sort Far Left Tube.

7 Install and load the corresponding tube on the cytometer.

This tube should contain sorted beads. Sorting instructions can be found in Chapter 5.

8 View the data on the global worksheet; make adjustments to gates, as needed.

Adjustments will also apply to the next tube that is viewed on the global worksheet. If you don't want to alter the global worksheet, record the data and make adjustments on the tube-specific worksheet.

9 Alt-click the current tube pointer to record data.

10 Click Next Tube to move the current tube pointer to the next tube in the Browser.

11 Repeat steps 7 through 10 for the remaining tubes in the experiment.

To view data on a tube-specific worksheet after recording, double-click the tube name in the Browser.

Sample QC Log

A sample QC log is provided on the following page. This log can be photocopied or used as a guide in designing your own QC log.

BD FACSAria QC Log

Sort Setup	Channel	FSC	SSC	1E	1D	1C	1B	1A	2B	2A	3B	3A
	Voltage											
Instrument Configuration	Filter											
	Parameter											
Date												
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											
	Mean/CV											

5

Sorting

You can program BD FACSDiva software to sort a specified number of particles from multiple populations into a variety of sorting devices, including tubes, plates, and slides. Collection devices are provided for sorting into 1-mL, 12 x 75-mm, and 15-mL tubes; hardware for sorting into plates and slides is available as an option. Up to four defined populations can be sorted into each tube, allowing up to 16 populations to be sorted at one time.

Any subsetted population can be used for sorting, including populations defined by quadrant gates, interval gates, or derived gates. A single sort population can be defined by up to eight gates.

The following topics are covered in this chapter:

- Setting Up for Sorting on page 150
- Determining the Drop Delay on page 154
- Sorting on page 158
- Setting Up for Sorting Into a Plate or Slide on page 163

Before you begin, you should be familiar with BD FACSAria operation using BD FACSDiva software. Review chapters 2 and 3, if needed.

Setting Up for Sorting

In general, do the following to set up for a sorting experiment. Each step is explained in more detail in previous or subsequent sections.

1 Install the appropriate-size nozzle.

As a general guideline, the nozzle opening should be at least three times the diameter of the particle to be sorted. See Changing the Nozzle on page 199.

2 Start up the instrument, the computer, and the stream.

See Instrument Startup on page 104.

When you turn on the stream, make sure it flows smoothly from the nozzle into the center of the waste aspirator. If you need help troubleshooting the stream, turn it off and go to Troubleshooting the Stream on page 212.

3 Choose a new sort setup option, if needed.

To change your current sort setup mode, choose High, Medium, or Low from the Sort > Sort Setup menu.

- High is designed for high-speed sorting with the 70-µm nozzle.
- Medium is designed for sorting cell lines or fragile cells with the 70-µm nozzle.
- Low is designed for single-cell sorting, ACDU sorting, or sorting large or fragile cells with the 100-µm nozzle.

For more information, see Sort Setup on page 92.

4 Adjust the Ampl. control to set the required drop breakoff.

Adjust the Drop 1 value, if needed, to match the generated value. For more information, see Setting Up the Breakoff on page 106.

If you need help troubleshooting the drop image, see Sorting Troubleshooting on page 217.

5 Turn on the Sweet Spot when the drop pattern is stable.

6 Check the laser delay and area scaling factors for your sheath pressure.

See Instrument Quality Control on page 113.

7 Optimize instrument settings for the sample to be sorted.

See Optimization of Instrument Settings on page 126.

8 Install the required collection device and set up the side streams.

See Setting Up for Bulk Sorting on page 152 or Setting Up for Sorting Into a Plate or Slide on page 163.

9 Calculate the drop delay.

See Determining the Drop Delay on page 154.

10 Use gating tools and subsetting methods to define the population(s) of interest.

Examples of gating analysis can be found in Analyzing Data on page 143 and in the BD FACSDiva software *Getting Started* guide.

11 Define a sort layout for the tube containing the defined sort populations and proceed with sorting.

See Sorting on page 158.

Setting Up for Bulk Sorting

This section describes how to set up the streams for two- or four-way sorting. For sorting using the ACDU option, see Setting Up for Sorting Into a Plate or Slide on page 163.

⚠ Any instrument surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing and gloves.

1 Install collection tubes in the required collection device.

Collection tube holders are available for 1-mL microtubes, 12 x 75-mm tubes, and 15-mL centrifuge tubes. For compatible tubes, see Labware on page 240.

2 Install the tube holder on the instrument.

Close the sort block door and open the sort collection chamber door, if needed. The sort block door must be closed in order to open the collection chamber door.

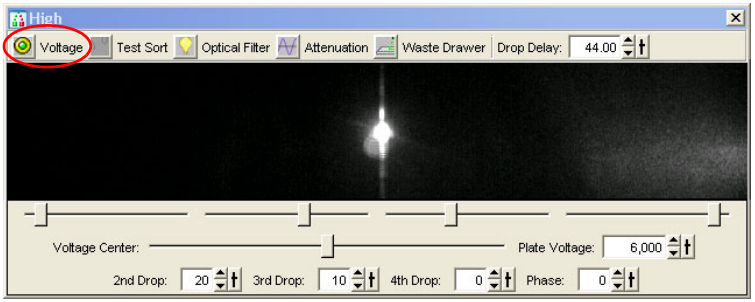
Slide the holder into the slotted fittings below the sort aspirator drawer, and close the sort collection chamber door.

⚠ Before installing the tube holder, ensure that an O-ring is installed in the groove on top of the tube holder. The O-ring minimizes the chance of aerosols escaping. It can be found in the accessory kit (Catalog No. 337897).

3 Turn on the deflection plates.

Click the Voltage control in the Side Stream window (Figure 5-1 on page 153). The voltage warning light illuminates, indicating that the plates are charged.

Figure 5-1 Turning on the deflection plates



Make sure the center stream image does not move after the plates are turned on. If the stream image is off-center, use the Voltage Center slider to re-center the stream.

Major movement of the center stream could indicate that the plates or area around the plates needs cleaning. See External Cleaning on page 174.

4 Turn on Test Sort and optimize the side streams.

Adjust the voltage sliders to view the required number of streams. If you cannot see a stream image or the image is dim, adjust the micrometer dial on the diode laser to better view the streams (Figure 1-17 on page 41).

- For four-way sorting, adjust the four slider controls until you see four streams.
- For two-way sorting, turn off the outer streams by moving the outer slider controls all the way to the center. Adjust the inner two slider controls until you see two streams.

5 Adjust the 2nd, 3rd, and 4th Drop settings to tighten the center stream and fine-tune the side streams, if needed.

Generally, the Sort Setup mode provides good starting values for these settings. Adjust the values only if needed to optimize the streams.

6 Open the aspirator drawer and aim the side stream(s) into the bottom of each collection tube.

- Open an experiment, if one is not already open, and create a sort layout for any of the tubes.
- In the Sort Layout window, click the Aspirator Drawer button to open the drawer ( ).
- Open the sort block door and aim each side stream into the bottom of a tube as you adjust the corresponding slider control in the side stream window.
- When you are satisfied with the side stream deflection, close the sort block door.

⚠⚠ A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the plate voltage is on. The plates remain energized even when the sort block door is opened.

7 Click the Voltage control ( ) to turn off the deflection plates.

Determining the Drop Delay

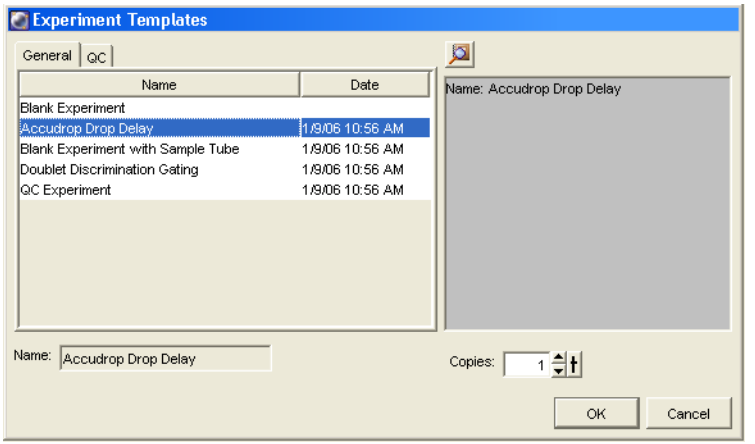
BD FACS Accudrop technology is used to determine the optimal drop delay setting for your sorting application. For more information, see Setting the Drop Delay on page 69. Before beginning this procedure, make sure the stream is stable and the Sweet Spot is on.

Setting Up the Experiment

The steps in this section show you how to set the drop delay using the Accudrop experiment template. Because no data is recorded, the experiment can be reused as often as you like.

1 Create an experiment from the *Accudrop Drop Delay* template.

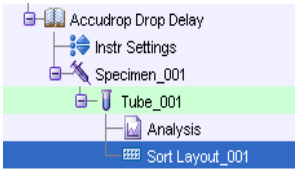
Choose Experiment > New Experiment. Select the Accudrop Drop Delay experiment and click OK.



2 Expand Specimen_001 and Tube_001.

3 Set the current tube pointer to Tube_001.

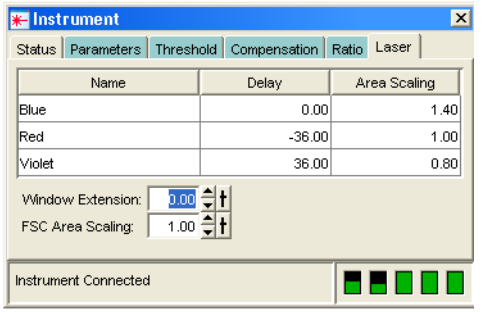
4 Open the sort layout by double-clicking it.



Sorting Beads to Determine the Drop Delay

1 Load a tube filled with a dilute suspension of Accudrop beads (approximately 2 drops of beads in 0.5 mL PBS).

2 In the Laser tab, set the window extension to zero.



3 Adjust the flow rate to achieve an event rate of 3,000–4,000 events per second.

☑ **Tip** If this cannot be achieved using a flow rate setting between 1 and 5, adjust the bead concentration.

4 Turn on the voltage in the Side Stream window; click Sort in the Sort Layout window.

5 Click Cancel at the Confirm prompt.

Confirm
Aspirator drawer is in WASTE position.
The drawer must be in SORT position
for drops to reach output device.
Click OK to move drawer to SORT position and start sort.
Click Cancel to start sort with drawer in WASTE position.
OK
Cancel

There is no need to collect the beads; when the drawer is closed, the beads are sorted to waste.

6 Click the Optical Filter control in the Side Stream window.

This control moves the emission filter that allows you to view the Accudrop beads in front of the lower camera. When the control is clicked, the image switches from a raw image to a processed (digitized) image. The two boxes indicate the region of the image where the left and center stream intensities are calculated during image processing; the numbers shown are percentages of the total intensity.

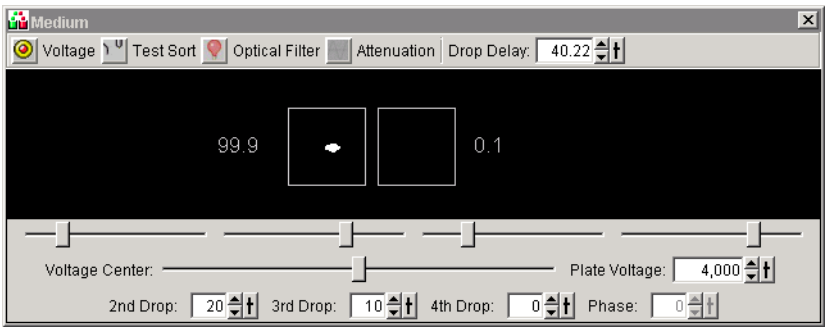
If the left side stream is not completely contained in the left region, adjust the voltage slider to place the stream in the center of the region.

7 Adjust the micrometer dial (Figure 1-17 on page 41) to obtain the brightest bead spot on the center stream.

8 Optimize the drop delay.

Adjust the Drop Delay value in 1-drop increments (Ctrl-click arrow control) to achieve close to 100% intensity in the left side stream. Wait a few seconds after each click for a complete response to the Delay change.

Figure 5-2 Sorting Accudrop beads in Initial mode

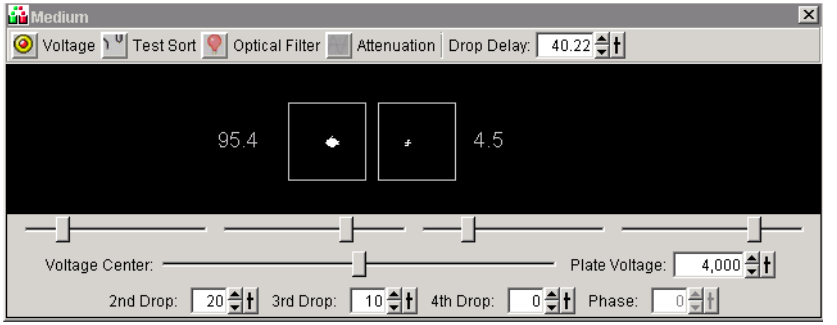


9 In the Sort Layout window, change the Precision mode to Fine Tune.

10 Optimize the drop delay.

Adjust the Drop Delay value in 0.03-drop increments (click arrow control) until the left side stream intensity is ≥90%. Wait a few seconds after each click for a complete response to the Delay change.

Figure 5-3 Sorting Accudrop beads in Fine-Tune mode



11 Click the Optical Filter control to move the emission filter away from the camera.

12 Reset the window extension to its original setting (typically, 2).

13 Turn off the deflection plates.

Sorting

Before beginning the sort, do the following:

- Perform the steps outlined in Setting Up for Sorting on page 150.
- Use gating tools and subsetting methods to define the population(s) of interest.

  Examples of gating analysis can be found in Analyzing Data on page 143 and in the BD FACSDiva software *Getting Started* guide.

  NOTICE Gates drawn on a biexponential scale can be used for sorting; however, the instrument will sort on a log scale. Therefore, a gate that crosses the zero boundaries will sort all events below zero into that gated population. This can cause a variance between the sort results and the statistical results in the software.

  NOTICE Snap-To gates cannot be used for sort gates.

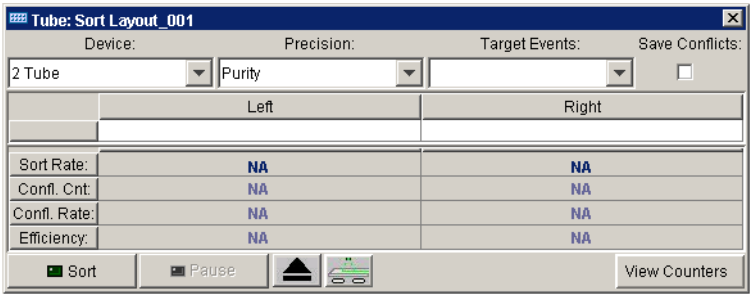
Setting Up the Experiment

☑ **Tip** When more than one drop is deflected in the same direction, residual charge from the first drop degrades the quality of the side streams. Thus, when four-way sorting or sorting into small wells where precise deflection is required, use the 4-Way Purity mode or choose a mode with a Yield Mask of zero. For more information, see Yield Mask on page 71.

1 In the Browser, right-click the tube containing the defined population(s) to be sorted and choose New Sort Layout.

Alternatively, if a global worksheet is shown, click the New Sort Layout button in the Workspace toolbar ( ). (In this case, the layout is added to the global worksheet.) By default, the 2-Tube Sort Layout appears.

2 Make appropriate entries in the Sort Layout window.



- Choose the collection device from the Device menu.
- Change the Sort Precision mode to Purity (two tubes), 4-Way Purity (four tubes), or Single Cell (plate or slide).
- Enter the number of Target Events by choosing a value from the drop-down menu or entering a number in the field.
- Select Save Conflicts if you are using a 2- or 4-Tube Sort Layout and you want to save conflicts.

- Select the sort location field(s) to be sorted into. Select multiple fields by dragging the mouse; select a row or column by clicking the row or column header.

- Add the required population(s) to each sort location field. If the Add menu doesn't appear after selecting the sort location field(s), right-click the selected fields to see the menu.

- Enter the number of Target Events and the population(s) for the remaining sort location fields, as needed.

- To display fewer counters in the Sort Layout window, click the View Counters button and deselect a menu option. The corresponding counter is hidden. (Only counters with a checkmark next to the name are displayed.)

Starting and Monitoring the Sort

1. Open the sort collection chamber door and install the collection tubes, plate, or slide.

2. Install the sample tube on the loading port and click Load.

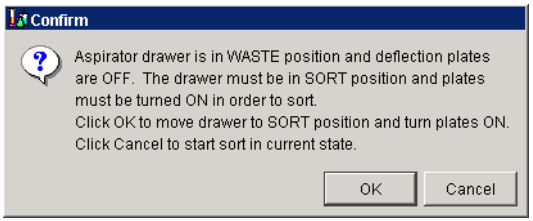
3. Adjust the required Flow Rate, if necessary.

4. (Optional) Turn on the deflection plates and open the aspirator drawer.

   The deflection plates turn off automatically each time a tube is unloaded. If you do not turn them back on before beginning a sort, a dialog box appears where you can turn on the plates and open the aspirator drawer by clicking OK.

5. Verify that the current tube pointer is indicating the appropriate tube in the Browser; click Sort.

6 Click OK if you are prompted to open the aspirator drawer or turn on the deflection plates.

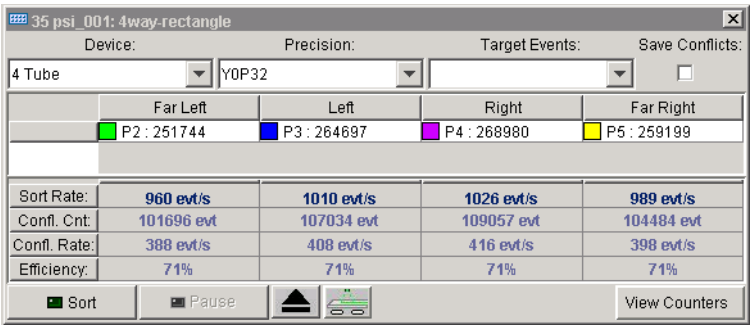

Confirm
Aspirator drawer is in WASTE position and deflection plates are OFF. The drawer must be in SORT position and plates must be turned ON in order to sort.
Click OK to move drawer to SORT position and turn plates ON.
Click Cancel to start sort in current state.
OK
Cancel

If you click Cancel at the message prompt, sorting will begin with the deflection plates off and the drawer closed. Thus, sort populations will be identified and counted, but no deflection (or sorting) will occur. If you sort with the drawer closed, events will be sorted to waste.

Tip Click Record Data to save data for the tube. Acquisition and sorting continue when the required number of events has been recorded.

Sorting continues until the required number of cells has been sorted. Acquisition stops and the drawer closes when sorting is complete. If the number of Target Events is set to Continuous, sorting continues until you manually stop sorting by clicking the Stop Acquiring or Sort button.

Monitor the sort progress from the Sort Layout window. The number of events sorted into each sort location appears in the corresponding field. The sort rate and sort conflict rate are displayed in the corresponding counter fields.



☑ **Tip** To pause during sorting, click the Pause button. Sort counts are retained when you restart sorting by clicking the Pause button again. If you need to unload the sample tube during sorting (ie, to add more sample), you can print a Sort Report to keep a record of the current sort counts. (The sort counts reset to zero when you restart sorting after loading a sample.)

☑ **Tip** When the Sweet Spot is on, sorting pauses automatically if the Drop 1 or Gap are out of range. This ensures that sorting occurs only under the proper breakoff conditions. If a more severe problem such as a clog is detected, the stream shuts off and sorting stops: the deflection plates shut off, the aspirator drawer closes, and the sample tube is unloaded. To clear a clogged nozzle, see Cleaning a Nozzle on page 203.

7 Print the Sort Report before you close the sort layout.

☑ **Tip** You can also export the report.

Setting Up for Sorting Into a Plate or Slide

The following sections describe how to set up for sorting into a plate or slide. For general guidelines, see Setting Up for Sorting on page 150.

Installing the Sorting Hardware

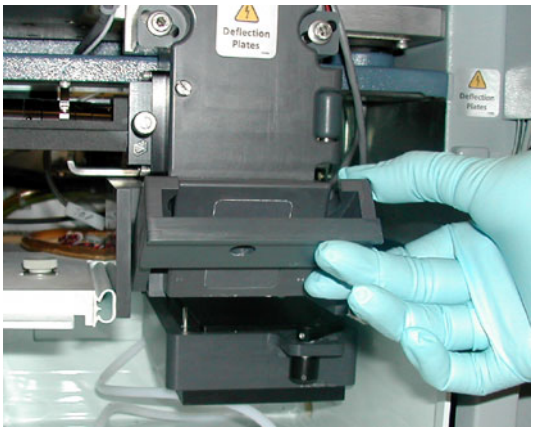
Any instrument surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing and gloves.

1 Install the splash shield below the aspirator drawer.

Close the sort block door and open the sort collection chamber door, if needed. The sort block door must be closed in order to open the collection chamber door.

Remove the tube holder, if one is installed, and tilt the splash shield to insert it into the slotted fittings below the sort aspirator drawer. Push the splash shield all the way in.

splash shield



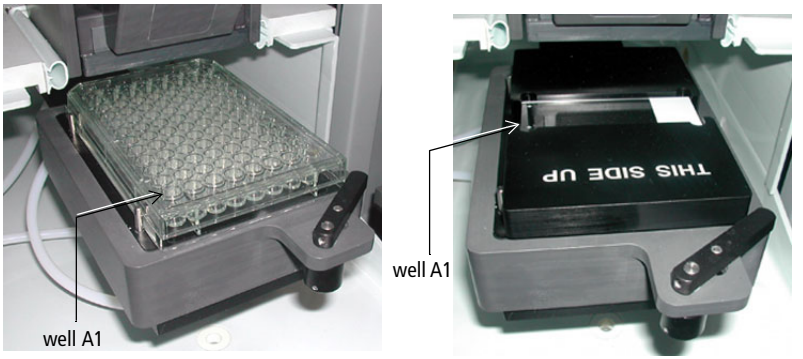
2 Click the Access Stage button to bring the ACDU stage to the front.

- Open an experiment, if one is not already open, and create a sort layout for any of the tubes.
- In the Sort Layout window, click the Access Stage button ( ) to move the stage to the front of the sort collection chamber.

3 Install the required collection device on the stage (Figure 5-4).

- If you are sorting into a plate, install the plate with well A1 toward the front of the stage.
- If you are sorting onto a slide, install the slide-adapter tray with the printed side up. If your slide has a frosted end, place the frosted end to the right.

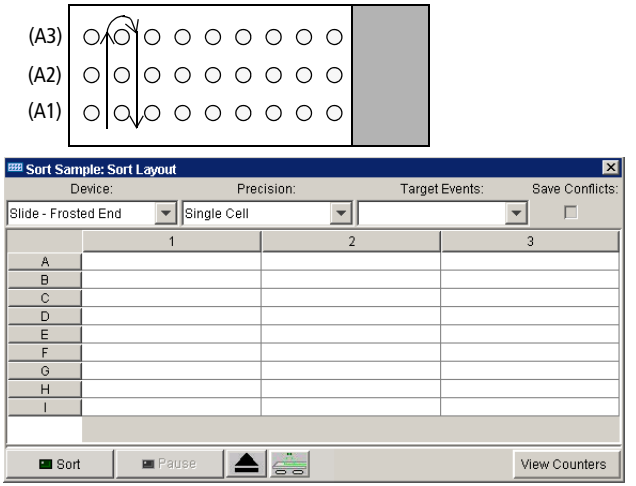
Figure 5-4 ACDU stage set up with plate (left) and slide (right)



Sorting starts on the front left corner of the device (A1 location), and proceeds from front to back, and then from the left to the right side of the device, sorting in a serpentine motion. Thus, for a plate, sorting proceeds from well A1–A12, B12–B1, C1–C12, and so on.

When sorting onto a slide, sorting proceeds in rows across the short end of the slide, and in columns along the long end of the slide. Make sure that you set up your sort layout accordingly. See Figure 5-5 on page 165.

Figure 5-5 Sort order on a slide



Setting Up the Stream

This section describes how to optimize side stream deflection and how to adjust the Home location.

When sorting into a plate or onto a slide, the stage is preprogrammed to move a set interval between wells on a plate or spots on a slide. The Home location is used as the starting point: the far left stream should hit the center of the well in the top left corner of a plate or the top left corner of a slide at the Home location.

Default Home location coordinates exist for each standard sort collection device: Falcon multiwell plates with 6, 24, 48, 96, and 384 wells, and standard or frosted-end slides. For other plate types, you will need to set the Home location. See Creating a Custom Device on page 168.

Use the following procedure to verify the Home location and adjust it.

1 Optimize the drop delay.

See Determining the Drop Delay on page 154.

2 Turn on the deflection plates.

Click the Voltage control ( ) in the Side Stream window. The voltage warning light illuminates, indicating that the plates are charged.

☑ **Tip** Make sure the center stream image does not move after the plates are turned on. If the stream image is off-center, use the Voltage Center slider to re-center the stream. Major movement of the center stream could indicate that the plates or area around the plates needs cleaning. See External Cleaning on page 174.

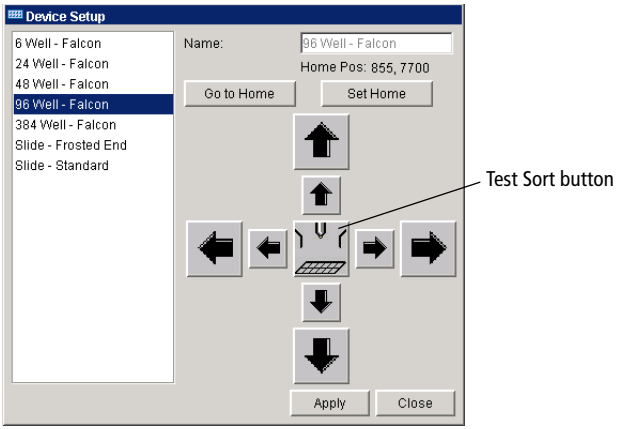
3 Turn on Test Sort and optimize the far left side stream; turn off Test Sort.

Adjust the far left slider for minimal deflection of the stream. The stream should be deflected just enough to clear the hole in the splash shield. Do not adjust the other sliders.

If you cannot see a stream image or the image is dim, adjust the micrometer dial on the diode laser (Figure 1-17 on page 41) to better view the streams.

4 Choose Sort > Home Device.

5 In the Device Setup dialog box, choose the collection device you are using and click Go to Home.



The stage moves to the preprogrammed Home position.

6 Double-click the Test Sort button to deposit a drop at the Home location.

7 Inspect the collection device to see where the drop was deposited.

If you need to move the stage to the front, close the Device Setup dialog box, and click the Access Stage control in the Sort Layout window.

8 Wipe the collection device dry, and place it back on the tray support.

If needed, click the Access Stage control to send the stage back and choose Sort > Home Position to access the Device Setup dialog again.

9 Adjust the Home location, if necessary.

Click the appropriate Arrow buttons to move the tray support as needed. Large arrows move the tray by five steps; small arrows move the tray by one step.

10 Repeat steps 6 through 9 until the drop is centered appropriately.

11 Click Set Home, then Close.

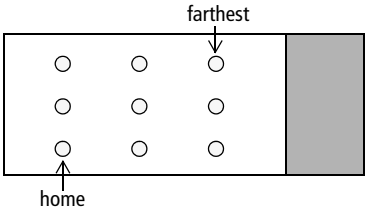
12 Click the Voltage control ( ) to turn off the deflection plates.

13 Proceed with Sorting on page 158.

Creating a Custom Device

You can program the ACDU stage to sort into any grid configuration. Create a custom device by entering the number of rows and columns and setting the Home and Farthest locations. BD FACSDiva software calculates the increment between rows and columns to determine the sort locations. The Home and Farthest locations for a 96-well plate are A1 and H12, respectively.

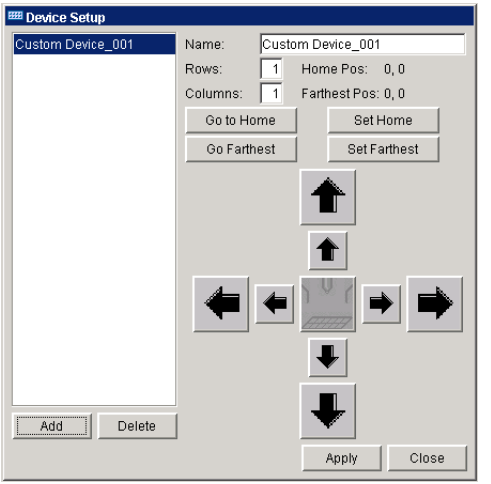
Figure 5-6 Home and Farthest location on 9-drop slide (example)



1 Choose Sort > Custom Devices.

2 Click the Add button in the Custom Devices dialog box.

A new device is added to the list of custom devices. By default, devices are named Custom Device_00*x*, where *x* is the next consecutively numbered device.



3 Select the text in the Name field and enter a new name.

4 Enter the number of sort location Rows and Columns.

A device can have up to 60 rows and 25 columns.

5 Use the Arrow keys and the Test Sort button to set the Home location; click Set Home.

See Setting Up the Stream on page 165 for details. There are no default values for custom devices, so greater initial adjustment with the Arrow keys is required.

6 Use the same procedure to set the Farthest location; click Set Farthest.

The Farthest sort location is the well or spot on the lower-right corner of the collection device.

7 Click Apply, then Close.

After you set the Home and Farthest locations, custom devices are listed in the Device drop-down menu in the Sort Layout window.

NOTICE Once custom devices are defined, you cannot change the number of rows and columns.

8 Click the Voltage control ( ) to turn off the deflection plates.

9 Proceed with Determining the Drop Delay on page 154 and Sorting on page 158.

Deleting a Custom Device

1. Choose Sort > Custom Devices.
2. Select the name of the custom device to be deleted in the Custom Devices dialog box.
3. Click Delete.

   The device is deleted from the Custom Device list, but is retained within any sort layouts where it was used.

6

Shutdown and Maintenance

The BD FACSAria instrument is designed to require minimum maintenance. However, to preserve the reliability of the instrument, you must regularly perform basic preventive maintenance procedures. This chapter explains routine maintenance procedures you should follow to keep your instrument in good condition.

Maintenance procedures are presented as follows:

- Daily Shutdown on page 172
- Scheduled Maintenance on page 175
- Unscheduled Maintenance on page 199

Daily Shutdown

Run the following procedures at the end of each day or when required:

- Fluidics Shutdown on this page
- External Cleaning on page 174

Fluidics Shutdown

At the end of each day the instrument is used, run fluidics shutdown to remove sheath fluid from the sheath path and rinse all lines with DI water. This prevents saline crystals from clogging the fluidics.

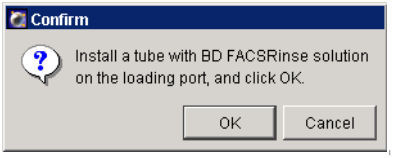
1. Unload your sample tube, if one is loaded.
2. Open the flow cell access door.
3. Turn off the stream.
4. Choose Instrument > Fluidics Shutdown, and click OK to begin.

5. Empty the waste and refill the DI water, if prompted to do so.

   See Setting Up the Fluidics Cart on page 108. If you disconnect the DI water tank for refilling, prime the system before continuing.

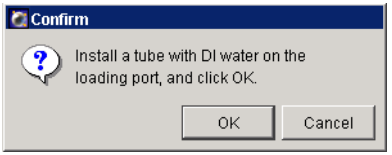
   The instrument starts the Daily Clean cycle. Daily Clean removes sheath from the lines and plenum and replaces it with DI water.

6 When prompted, install a tube containing approximately 3 mL of cleaning fluid such as BD FACSRinse™ solution (BD Catalog No. 340346), and click OK.



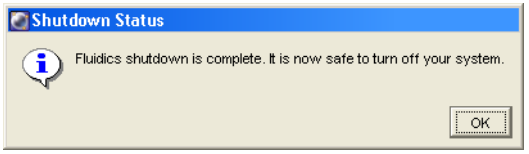
The instrument loads the tube and cleans the cuvette with the cleaning solution. A progress message appears during the cleaning process; click OK to dismiss the message.

7 When prompted, install a tube containing approximately 3 mL of *clean* DI water; click OK.



The instrument loads the tube and rinses the cuvette with DI water. A progress message appears during the cleaning process; click OK to dismiss the message.

8 Click OK when you see a message informing you the system can be turned off.



9 Turn off the instrument main power; quit BD FACSDiva software and shut down the computer.

During shutdown, you will normally hear a hiss, caused by condensed water discharging from the fluidics cart pumps.

Turn off power to the cytometer at least once a day. If your laboratory runs the cytometer continuously and does not shut down at the end of the day, switch on the Auxiliary Air Supply for 15 seconds every 8 hours (every 4 hours in an extremely humid climate). Doing this empties the cart condensation trap and prevents excess moisture from overflowing the trap or causing cart damage.

10 (Optional) Remove the nozzle and allow it to air dry.

External Cleaning

To keep the system free from salt buildup, wipe down all instrument surfaces that have been exposed to sheath fluid. Clean surfaces with a cloth dampened with a 10% bleach solution, followed by DI water.

All instrument surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning instrument surfaces. Wear suitable protective clothing and gloves.

To prevent shock, turn off the plate voltage before cleaning on or around the deflection plates. To prevent arcing (sparking), make sure the plates are completely dry before you turn the plate voltage back on.

The following surfaces should be inspected and cleaned when necessary:

- inside the sort block
- deflection plates

Scheduled Maintenance

For optimal instrument functioning, perform the following procedures according to the recommended maintenance schedule.

Procedure	Recommended Frequency
Internal Cleaning on this page	See Table 6-1 on page 176
Purging Filters on page 192	Weekly
Changing the disposable waste cap (see Emptying the Waste on page 110)	Monthly
Changing Fluid Filters on page 193	Every 3–6 months
Changing the Sample Lines on page 194	Every 4–6 months, or when required
Changing Air Filters on page 197	Every 6–12 months, depending on instrument use and the quality of the air
Changing the Bubble Filter on page 197	Every 6 months or when increased debris appears in the FSC vs SSC dot plot

Internal Cleaning

BD FACSDiva software includes four pre-programmed cleaning modes that can be used singly or in combination to provide the required level of cleaning.

The following sections describe what actions are taken when you choose the indicated command from the Instrument > Cleaning Modes menu. See Table 6-1 on page 176 for an overview of each mode.

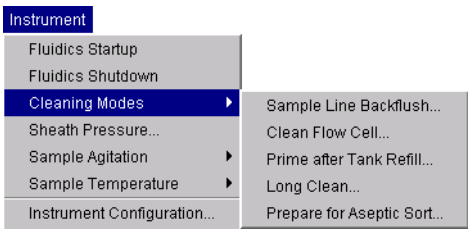


Table 6-1 Instrument cleaning modes

Cleaning Mode	Summary	Frequency
Sample Line Backflush on page 177	Flushes the sample line with sheath fluid	After running samples with adherent cells or dye
Clean Flow Cell on page 178	Cleans the sample path and the flow cell with a decontaminant	When indicated by distorted scatter, high CVs
Prime After Tank Refill on page 179	Primes the fluid lines for the designated fluid(s)	When a fluidics line is unplugged to refill a tank
Long Clean on page 180	Decontaminates the internal sheath path with bleach or ethanol and rinses it with DI water	Monthly
Clean Bulk Injection Chamber on page 185	Decontaminates the sample injection chamber with ethanol	Monthly
Prepare for Aseptic Sort on page 187	Decontaminates the complete sheath path with ethanol and rinses it with DI water	When needed before aseptic sorting

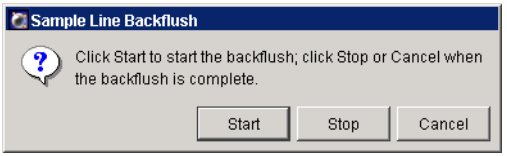
Some of the internal cleaning modes require that you turn off the stream. Remember to always block the lasers before you turn off the stream. Running the lasers without the stream can degrade the performance of the cuvette flow cell.

Sample Line Backflush

After a sample tube is unloaded, the sample line tubing within the sample injection chamber is automatically flushed inside and out with sheath fluid to eliminate potential sample carryover. Use the Sample Line Backflush command to perform additional backflushing of the inside of the sample line after a tube unloads. Perform the Sample Line Backflush when you observe sample carryover or after running samples with adherent cells or dye.

NOTICE Keep the stream running while performing the backflush.

1. Choose Instrument > Cleaning Modes > Sample Line Backflush.
2. Click Start to start the backflush.


Sample Line Backflush
Click Start to start the backflush; click Stop or Cancel when the backflush is complete.
Start
Stop
Cancel

3. Click Stop to stop the backflush, or click Cancel to stop the backflush and close the dialog box.

   The backflush does not stop automatically.

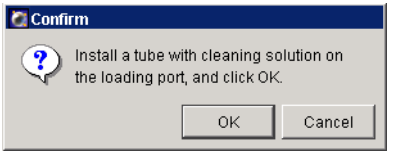
Clean Flow Cell

Use the Clean Flow Cell command to run a tube of cleaning fluid such as BD FACSRinse solution through the sample line and flow cell, without sheath running.

NOTICE After the procedure is complete, the cleaning fluid remains in the sample line and flow cell until the stream is restarted.

Note that the Clean Flow Cell procedure is run automatically during fluidics shutdown. You can repeat the procedure when poor optical performance indicates that additional cleaning is needed.

1. Open the flow cell access door and turn off the stream.
2. Remove the nozzle.
3. Choose Instrument > Cleaning Modes > Clean Flow Cell.
4. When prompted, install a tube containing approximately 3 mL of cleaning fluid (such as BD FACSRinse solution), and click OK.

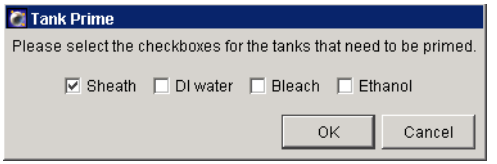


   The instrument loads the tube and cleans the cuvette with the cleaning solution. A message appears when cleaning is finished.
5. Click OK when the completion message appears.
6. Replace the nozzle.
7. Wait a few minutes and then restart the stream to flush the cleaning solution from the flow cell.

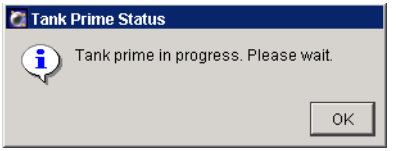
Prime After Tank Refill

Use the Prime After Tank Refill command to prime the fluid lines if a fluidics container was disconnected for refilling.

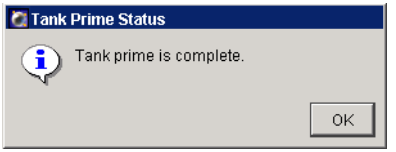
1. Open the flow cell access door and turn off the stream.
2. Choose Instrument > Cleaning Modes > Prime After Tank Refill.
3. Select the checkboxes for the tanks that were refilled; click OK.



The instrument proceeds with priming the specified tanks. The bleach and ethanol tanks are primed before the DI tank is primed. A progress message appears while the tanks are being primed; click OK to dismiss the message, if you would like.



4. Click OK when you see a message informing you that the tank prime is complete.



Long Clean

Use the Long Clean command to decontaminate, with bleach or ethanol, the internal sheath path from the fluid line (starting at the outlet of the fluid selection valves in the fluidics cart) to the nozzle outlet. After decontamination, the lines are rinsed with DI water.

There are three Long Clean options: Long Clean with Bleach, Long Clean with Ethanol, and Clean Bulk Injection Chamber. Use the Bleach option to remove adhesive dyes such as PI, acridine orange, or thiazole orange, or when you want to decontaminate the sample lines. Use the Ethanol option to decontaminate the lines with ethanol. The remaining option is described in Clean Bulk Injection Chamber on page 185.

Consult with your BD representative before running any other cleaning solutions.

All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Use 10% bleach to decontaminate the sheath path. Flushing the lines with 10% bleach is the only procedure recommended by BD Biosciences for decontaminating the instrument.

This procedure can take 10–40 minutes, depending on how long you run the stream to remove trace decontaminants.

1. Ensure fluid levels are adequate; empty the waste container if it is full.

   For example, if you are performing a bleach cleaning procedure, make sure the bleach tank is full. The long clean requires approximately 90 mL of cleaning fluid and 110 mL of DI water.

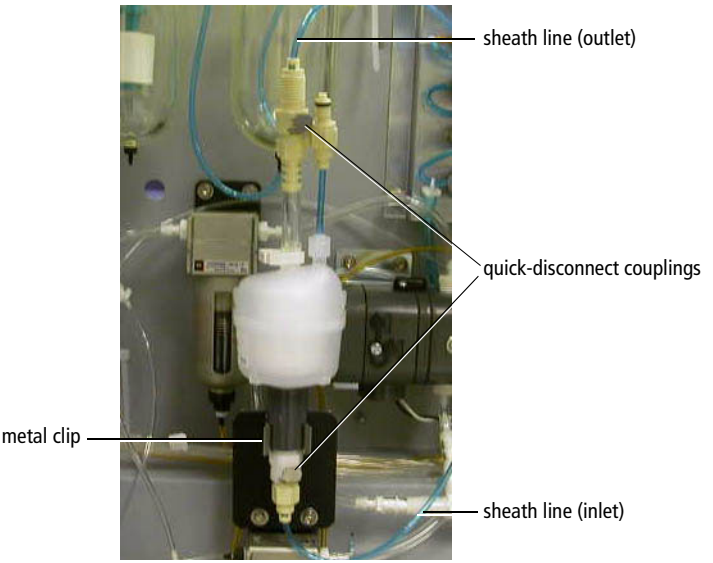
2. Open the flow cell access door and turn off the stream.

3. Bypass the bubble filter.

To prevent damage to the bubble filter, do not run bleach or ethanol through the filter. Bypass the bubble filter as follows.

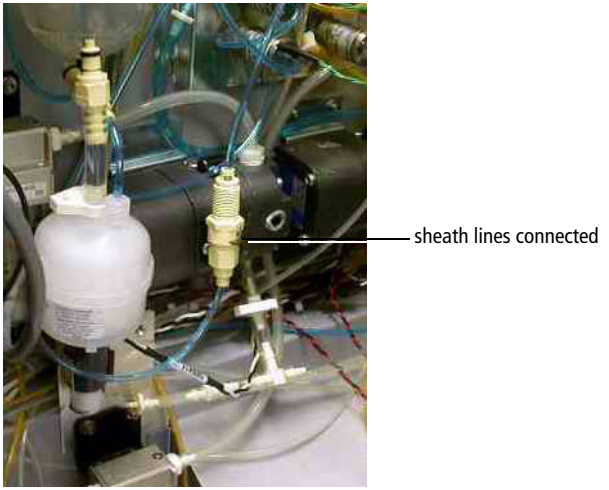
- Reduce the sheath pressure to 10 psi.
- Using a paper towel to cover the quick-disconnect coupling, disconnect the sheath line from the filter outlet tubing at the top of the filter:

Figure 6-1 Disconnecting sheath line above and below filter



- Using a paper towel to cover the quick-disconnect coupling, disconnect the sheath line from the filter inlet coupling at the bottom of the filter (Figure 6-1).
- Connect the two sheath line couplings together (Figure 6-2 on page 182).
- Set the sheath pressure back to its original setting.

Figure 6-2 Bypassing the bubble filter



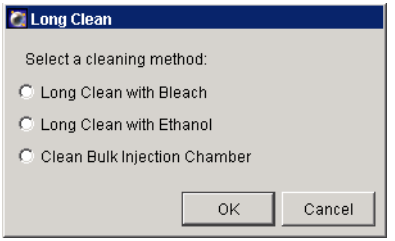
⚠ Make sure you reconnect the bubble filter when you are finished running the cleaning mode.

4 Choose Instrument > Cleaning Modes > Long Clean.

If you have not already turned off the stream, a message appears reminding you to do so.

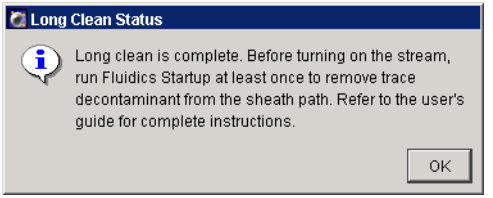
5 Select a Long Clean option; click OK.

Choose from the following:



For the bulk injection chamber option, see Clean Bulk Injection Chamber on page 185.

6 Wait 8–10 minutes for the cleaning cycle to finish; click OK to dismiss the completion message.


Long Clean Status
Long clean is complete. Before turning on the stream, run Fluidics Startup at least once to remove trace decontaminant from the sheath path. Refer to the user's guide for complete instructions.
OK

If the completion message does not appear after 10 minutes, verify that there are no error messages in the Status tab of the Instrument window. If the cleaning mode fails, see Fluidics Troubleshooting on page 229.

7 (Optional) Remove the plenum reservoirs and wipe them down with 10% bleach, followed by DI water.

BD Biosciences recommends that you remove the plenum reservoirs only for the most stringent decontamination. You do not need to perform this step each time you run a Long Clean.

For instructions, see Cleaning the Plenum Reservoirs on page 184.

8 Run fluidics startup.

Fluidics startup removes cleaning fluid from the plenum tanks and fluid lines and replaces it with sheath fluid.

The following steps are not required, but are recommended for removing residual decontaminant after performing a Long Clean with bleach or ethanol. This will ensure a safe environment for biological samples.

9 When fluidics startup is complete, run fluidics shutdown.

10 When fluidics shutdown is complete, run fluidics startup again.

11 (Optional) When startup is complete, turn on the stream and let it run for 20–30 minutes.

Cleaning the Plenum Reservoirs

After you run a Long Clean with bleach or ethanol, you can clean the plenum reservoirs by wiping them down with bleach or DI water. Use the following procedure to remove and clean the reservoirs.

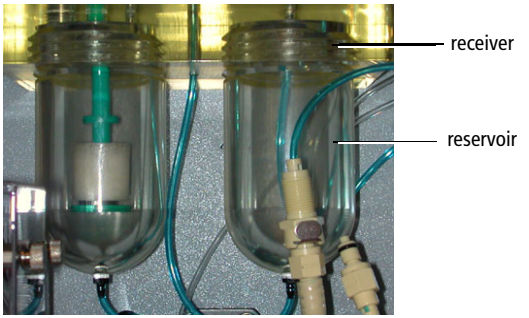
⚠ To prevent damage to the plenum reservoirs, do not autoclave them.

1 Turn off the instrument main power.

2 Open the side door and verify that the plenum reservoirs are empty.

3 Place absorbent paper under the plenum reservoirs and remove each one by unscrewing it from its receiver.

NOTICE The reservoirs must be reinstalled in the same locations. To keep track, use an indelible marker to label them *Left* and *Right* when you remove them.



4 Pull off the tubing connected to each reservoir.

5 Wipe the reservoirs with 10% bleach, followed by DI water.

After wiping, dry them with a clean, lint-free cloth.

6 Clean the float and receiver for each plenum reservoir.

Use a spray bottle to clean the floats. Use a cloth soaked with bleach or ethanol to wipe down the receivers.

⚠ The floats are fragile. Handle them with care. Do not spray ethanol into the instrument behind or below the plenum reservoirs.

Dry each float and reservoir with a clean, lint-free cloth.

7 Reinstall the plenum reservoirs in their original locations.

Screw each reservoir to its receiver, making sure that the O-rings are seated properly within each plenum reservoir. Reattach the tubing to the bottom of each reservoir.

Clean Bulk Injection Chamber

Use the Clean Bulk Injection Chamber option to flush the injection chamber with DI water and ethanol.

Allow 5–10 minutes to complete the cleaning procedure.

1 Ensure fluid levels are adequate; empty the waste container if it is full.

This procedure requires approximately 250 mL each of DI water and ethanol.

2 Open the flow cell access door and turn off the stream.

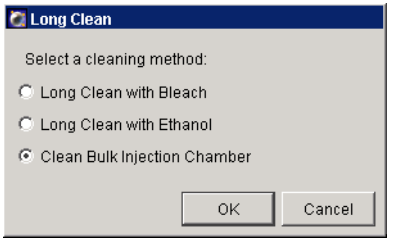
3 Remove the tube holder from the loading port.

It can be cleaned separately by wiping it down with ethanol, if needed.

4 Choose Instrument > Cleaning Modes > Long Clean.

If you have not already turned off the stream, a message appears reminding you to do so.

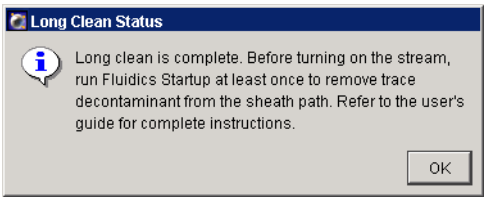
5 Select the Clean Bulk Injection Chamber option; click OK.



During the next 4–5 minutes, the instrument performs the following:

- closes and seals the bulk injection chamber
- fills the chamber with DI water, and flushes the chamber
- fills the chamber with ethanol, and flushes the chamber
- air-dries the chamber, and then opens it

A message appears when cleaning is complete.


Long Clean Status
Long clean is complete. Before turning on the stream, run Fluidics Startup at least once to remove trace decontaminant from the sheath path. Refer to the user's guide for complete instructions.
OK

6 Click OK to dismiss the completion message.

7 Use a clean, dry cloth to remove residual ethanol from the loading port.

8 Before using the system, run fluidics startup.

Prepare for Aseptic Sort

Use the Prepare for Aseptic Sort command when you want to decontaminate the entire sheath path with 70% ethanol or 10% bleach, from the fluid line starting at the sheath inlet port in the fluidics cart to the nozzle outlet. For the most complete decontamination, autoclave the sheath container and install a new sheath filter before sorting. Autoclave the sheath container at 125°C and 15 psi for 30 minutes with a 7.5-minute warm-up and shut-down cycle.

NOTICE To decontaminate the sheath probe, use 10% bleach. Do not autoclave the probe.

The plenum reservoirs can also be removed and cleaned with bleach before you run this cleaning mode, if required. To do so, run a Long Clean with ethanol or bleach as described in Long Clean on page 180, and then clean the plenum reservoirs as described on page 184.

This procedure takes 10–40 minutes, depending on how long you run the stream to remove trace decontaminants. It consists of the following four tasks:

- Preparing the Instrument
- Bypassing the Bubble Filter on page 189
- Cleaning the Lines with Ethanol or Bleach on page 190
- Replacing the Ethanol or Bleach with Sheath Fluid on page 191

Make sure you perform the entire procedure.

Preparing the Instrument

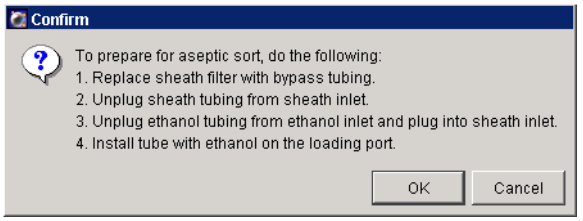
1. Ensure fluid levels are adequate; empty the waste container if it is full.

   This procedure requires approximately 250 mL of bleach or ethanol.

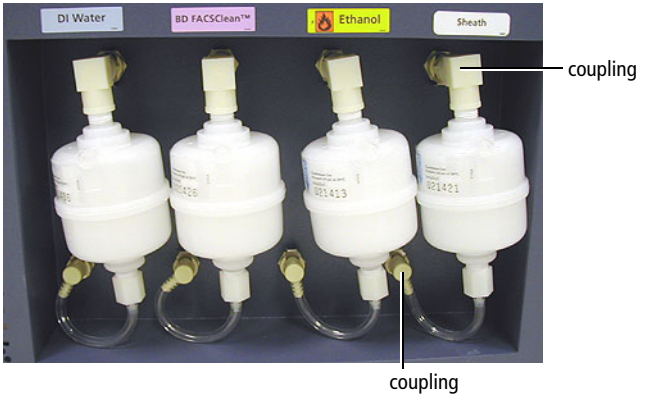
2. Open the flow cell access door and turn off the stream.

3. Choose Instrument > Cleaning Modes > Prepare for Aseptic Sort.

A dialog box appears with instructions on how to set up for cleaning.

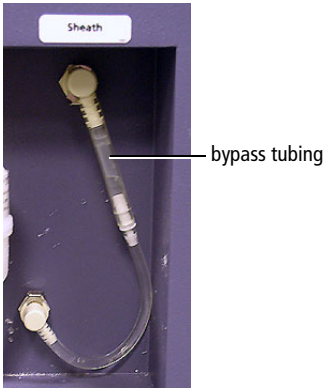

Confirm
To prepare for aseptic sort, do the following:
1. Replace sheath filter with bypass tubing.
2. Unplug sheath tubing from sheath inlet.
3. Unplug ethanol tubing from ethanol inlet and plug into sheath inlet.
4. Install tube with ethanol on the loading port.
OK
Cancel

4 Remove the sheath filter by pressing the tabs on each quick-disconnect coupling.



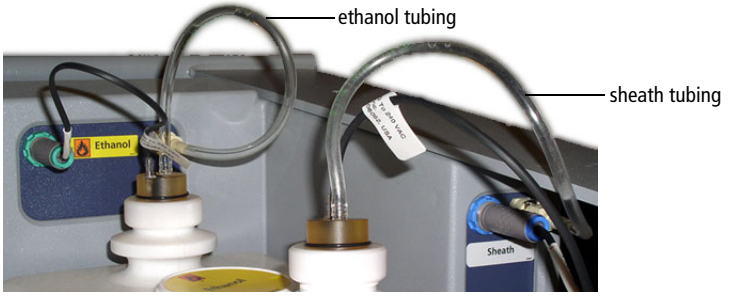
5 Install bypass tubing in place of the sheath filter.

Push the tubing coupling into each quick-disconnect port until you hear a click.



6 Detach the sheath tubing and the ethanol or bleach tubing from their respective ports on the fluidics cart.

Figure 6-3 Sheath and ethanol ports



7 Plug the ethanol or bleach tank quick-disconnect into the sheath port.

Bypassing the Bubble Filter

⚠ Do not run bleach or ethanol through the bubble filter, as it will damage the filter. Bypass the bubble filter as follows.

1 Reduce the sheath pressure to 10 psi.

2 Using a paper towel to cover the quick-disconnect coupling, disconnect the sheath line from the filter outlet tubing at the top of the filter (Figure 6-1 on page 181).

3 Using a paper towel to cover the quick-disconnect coupling, disconnect the sheath line from the filter inlet coupling at the bottom of the filter (Figure 6-1 on page 181).

4 Connect the two sheath line couplings together (Figure 6-2 on page 182).

5 Set the sheath pressure back to its original setting.

⚠ Make sure you reconnect the bubble filter when you are finished running the cleaning mode.

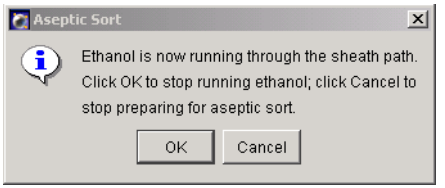
Cleaning the Lines with Ethanol or Bleach

1 Install a tube containing ethanol or bleach onto the loading port; click OK.

A progress message appears. During the next 3–5 minutes, the instrument performs the following:

- primes all fluid lines with ethanol/bleach
- turns on the stream
- loads the ethanol/bleach tube and starts running it

Once the instrument is running ethanol or bleach through all lines, a message appears.


Aseptic Sort
Ethanol is now running through the sheath path.
Click OK to stop running ethanol; click Cancel to
stop preparing for aseptic sort.
OK
Cancel

2 Let the ethanol/bleach run for 10 minutes, unless your application requires longer for decontamination; **click OK** when finished.

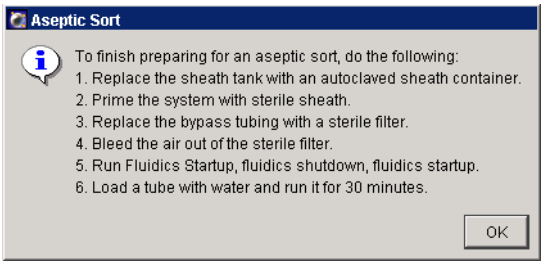
Do not run ethanol/bleach longer than is necessary to decontaminate.

NOTICE Click Cancel only if you want to cancel the cleaning mode. Do not click Cancel to stop running ethanol/bleach.

The instrument stops running the ethanol/bleach sample, unloads the tube, and stops the stream.

Replacing the Ethanol or Bleach with Sheath Fluid

Now that all fluid lines have been cleaned, the system prompts you to install an autoclaved sheath container on the fluidic cart. Follow these steps to complete the cleaning procedure.


Aseptic Sort
To finish preparing for an aseptic sort, do the following:
1. Replace the sheath tank with an autoclaved sheath container.
2. Prime the system with sterile sheath.
3. Replace the bypass tubing with a sterile filter.
4. Bleed the air out of the sterile filter.
5. Run Fluidics Startup, fluidics shutdown, fluidics startup.
6. Load a tube with water and run it for 30 minutes.
OK

1 At the prompt, detach the ethanol or bleach line from the Sheath port on the fluidics cart and connect it to the ethanol or bleach port (Figure 6-3 on page 189).

2 Connect an autoclaved sheath container to the Sheath port.

☑ **Tip** To prevent contamination, clean the sheath probe and tubing with 10% bleach. Fill the container with sterile sheath fluid.

3 Prime the system with sterile sheath fluid.

- Click OK to dismiss the instruction message, and choose Instrument > Cleaning Modes > Prime After Tank Refill.
- Select the Sheath checkbox, and click OK.

4 When the prime is finished, remove the bypass tubing and install a new sheath filter in its place.

5 Open the bleeder valve on top of the filter and wait for fluid to seep out of the bleeder valve; close the valve.

See Purging Filters on page 192.

6 Run fluidics startup.

For additional decontamination, run fluidics shutdown when the system is ready and then repeat fluidics startup again.

☑ **Tip** Be sure to use sterile water for the shutdown procedure.

7 Install a tube of sterile DI water on the loading port; load the tube and run it for 10 minutes.

8 (Optional) Run the stream for an additional 10–20 minutes.

NOTICE Additional time might be needed to allow the stream and breakoff to stabilize.

Purging Filters

Once a week, purge air from all fluid filters by opening the bleeder valve on the top of each filter in turn. This ensures that the filters will not dry out.

1 Unscrew the bleeder valve on the top of the filter.

2 Wait until fluid seeps out through the valve.

3 Close the valve.

4 Wipe up any excess fluid that might have dripped onto the fluidics cart.

Changing Fluid Filters

BD Biosciences recommends changing the fluid filters every 3–6 months, or when increased debris in an FSC vs SSC plot indicates that the sheath filter needs to be replaced. Spare filters are included with the accessory kit.

1 Remove the filter by pressing the tabs on each quick-disconnect coupling.

2 Install the new filter and connect the quick-disconnect couplings.

☑ **Tip** Write today's date on the filter so you will know when to replace it.

3 Open the bleeder valve on top of the filter and leave it open until fluid seeps out through the valve.

4 Close the valve.

5 Wipe up any excess fluid that might have dripped onto the fluidics cart.

Changing the Sample Lines

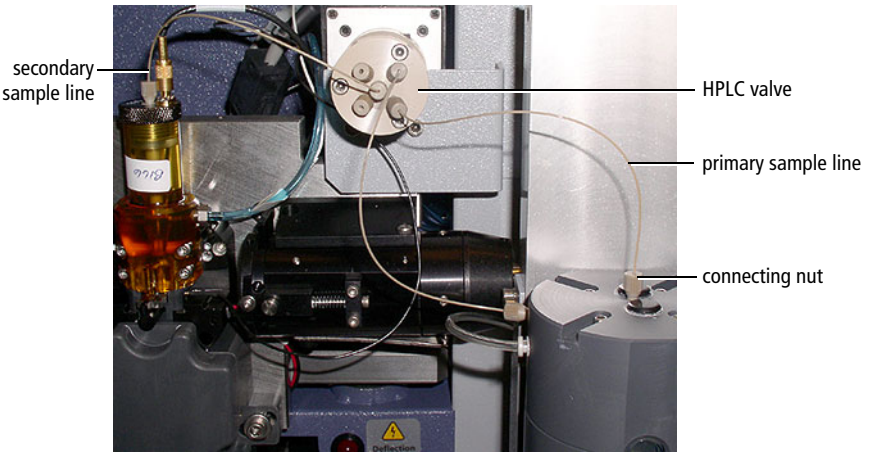
The primary sample line between the sample injection chamber and the HPLC valve should be changed every 4–6 months or when decreased event rates indicate that the sample line might be clogged. The secondary sample line between the HPLC valve and the cuvette flow cell needs changing only when it is kinked or clogged.

To withstand the high pressures generated by the BD FACSAria flow cytometer, the sample lines are attached at each end using a two-piece compression fitting, where a cone-shaped ferrule is compressed onto the tubing as the connecting nut is tightened.

To replace the tubing, you will need a length of replacement tubing from the accessory kit. The 12-in length of tubing is used for the primary sample line; the 7-in length is used for the secondary line (Figure 6-4). The ferrules and connecting nuts can be reused when the tubing is replaced.

⚠ All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle used tubing and fittings as if capable of transmitting infection. Wear suitable protective clothing and gloves.

Figure 6-4 Sample lines and HPLC valve



Use the following steps to replace the tubing. The same procedure can be used to replace either the primary or secondary sample line.

1. Turn off the stream and lasers (if needed); make sure the sample injection chamber is in the unload position.

2. Unscrew the connecting nuts on each end of the tubing, and pull out the sample line.

3. Ensure that a cone-shaped ferrule is attached to each end of the sample line.

   Figure 6-5 on page 196 shows an example of a ferrule. If the ferrule was left behind in the injection chamber fitting, HPLC valve, or flow cell fitting, screw the tip of the ferrule-removal tool (included in the accessory kit) into the top of the ferrule and pull the ferrule straight out.

   After using the tool, you might need to replace the ferrule. If the ferrule is damaged, spares are included in the accessory kit.

ferrule-removal tool

4. Slide the ferrule and nut off each end of the sample line.

☑ **Tip** Handle the ferrule with care; it is small and easily lost.

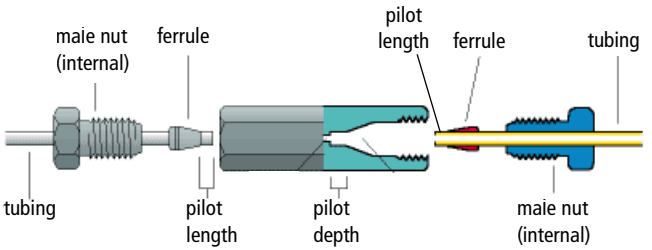
5. Slide the nut and then the ferrule onto each end of the new sample tubing.

   - If you are replacing the primary sample line, leave approximately 0.1 in. (0.25 cm) of tubing extending out of the ferrule on the HPLC end, and approximately 5 in. (12.7 cm) of tubing extending out of the sample injection chamber end. (This length can be adjusted depending on the depth of your sample tube.)

   - If you are replacing the secondary sample line, leave approximately 0.1 in. (0.25 cm) of tubing extending out of the ferrule on both ends.

   This length is referred to as the pilot, as shown in Figure 6-5 on page 196.

Figure 6-5 Components of a compression fitting (diagram courtesy of Valco Instruments Co. Inc.)



6 Insert the pilot tubing into its fittings, ensuring that the tubing reaches the intended pilot depths.

⚠ Try not to bend the primary sample line during insertion.

- For the primary sample line, insert the long end into the sample injection chamber fitting. Push the tubing from the top until it is slightly above the bottom of the chamber viewing window. Hand-tighten the nut on top of the chamber, and then insert the short end into the HPLC valve.
- For the secondary sample line, insert one end into the HPLC valve, and the other end into the cuvette flow cell fitting.

⚠ Within the HPLC valve and the cuvette flow cell fitting, make sure the pilot is seated flush against the pilot depth. Dead volume between the pilot and the pilot depth can lead to sample carryover or leaking.

7 While pushing the tubing in, hand-tighten each nut firmly.

⚠ Do not overtighten the nut and do not use tools. Over-tightening the nut can kink or damage the tubing so it would have to be replaced again.

8 Check the fitting connections to make sure they are not leaking.

Turn on the stream, load a tube of water, and make sure none of the fittings are leaking. If needed, unload the tube, turn off the stream, and tighten the fittings. After tightening, if leaking still occurs, replace the ferrule.

⚠☣ Make sure all compression fittings are securely tightened. If any fitting is loose, the tubing could detach during high-pressure operation, exposing the operator to potentially biohazardous sample spray.

9 Verify the length of the sample line.

The sample line should not bow or bend when a tube is loaded. If you need to adjust the length, unscrew the nut on top of the sample injection chamber, adjust the length, and tighten the nut again.

Changing Air Filters

The BD FACSAria instrument has two air filters: one in the sort collection chamber door and one in the side door.

- To change the filter in the sort collection chamber door, slide out the old filter and slide in the new filter. Refer to the documentation provided with the Aerosol Management Option for more information.

- To change the air filter in the side door, turn the pegs at each corner of the filter and remove the old filter. Install a new filter and turn the pegs to hold the filter in place. Replace the filter annually to semi-annually, depending on the quality of the air in the laboratory.

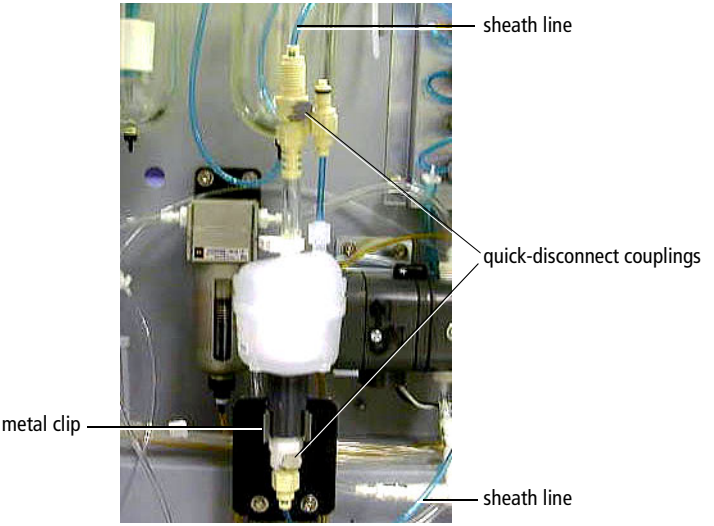
Changing the Bubble Filter

Change the bubble filter every 6 months or when increased debris appears in the FSC vs SSC dot plot.

1 Reduce the sheath pressure to 10 psi.

2 Using a paper towel to cover the quick-disconnect coupling, disconnect the sheath line from the filter outlet tubing at the top of the filter (Figure 6-6).

Figure 6-6 Removing the bubble filter



3 Using a paper towel to cover the quick-disconnect coupling, disconnect the sheath line from the filter inlet coupling at the bottom of the filter (Figure 6-6).

4 Pull the filter from the metal clip at the base of the filter.

5 Install the new filter.

Snap the filter into the metal clip and connect the quick-disconnect couplings at the top and bottom of the filter.

6 Set the sheath pressure back to its original setting.

7 In BD FACSDiva software, run fluidics startup.

8 During fluidics startup, gently tap the bubble filter to dislodge air bubbles.

9 Purge the bubble filter as described in Purging the Bubble Filter on page 208.

Unscheduled Maintenance

There are several instrument components that should be cleaned periodically or checked for wear and replaced if necessary. See the indicated sections for the following maintenance procedures.

Procedure	Recommended Frequency
Changing the Nozzle on this page	As needed when running different size particles
Adjusting the Nozzle Orifice on page 201	When satellite drops are not merging properly
Cleaning a Nozzle on page 203	When stream irregularities indicate the nozzle is clogged
Cleaning the Camera Windows on page 205	When smudges appear in the Breakoff or Side Stream windows
Cleaning Optical Filters on page 207	As needed when changing a filter
Purging the Bubble Filter on page 208	As needed to keep the flow cell clear of bubbles

Changing the Nozzle

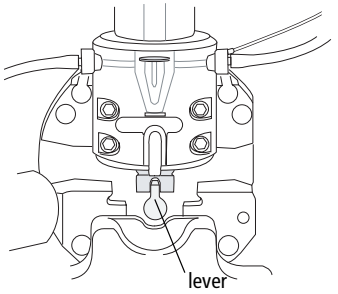
As a general guideline, the nozzle opening should be at least three times the diameter of the particle to be sorted. Two sizes of nozzles are provided with your instrument: 70- and 100-µm nozzles. The size is marked on the nozzle.

Any instrument surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing and gloves.

1. Open the flow cell access door, and turn off the stream.

2. Turn the nozzle-locking lever to the left, and pull the nozzle out of the cuvette flow cell (Figure 6-7 on page 200).

Figure 6-7 Nozzle-locking lever



☑ **Tip** To avoid losing the O-ring, hold the nozzle steady and pull it straight out without tilting it from side to side. If you do not see the O-ring on the nozzle, it could be stuck to the cuvette. To locate and remove the O-ring, wet the wooden end of a cotton swab and use it to wipe the inside of the nozzle well.

3 Transfer the nozzle O-ring to the new nozzle or set it aside with the nozzle.

4 Carefully insert the new nozzle into the flow cell.

Use the magnifying glass in the accessory kit to make sure an O-ring is installed in the groove at the end of the nozzle. Insert the nozzle at a slight downward angle to avoid contact between the O-ring and the cuvette. Push it gently all the way forward without rocking it from side to side.

5 Turn the nozzle-locking lever clockwise to the 12:00 position.

6 Turn on the stream and make sure it runs through the nozzle properly.

NOTICE After changing the nozzle, you might need to adjust the angle of the sort block to re-center the stream in the aspirator. To do so, loosen the adjustment screws on either side of the deflection plates and rotate the sort block (see Figure 1-11 on page 32). Tighten the screws when the stream is centered in the aspirator. For further assistance, see Troubleshooting the Stream on page 212.

Adjusting the Nozzle Orifice

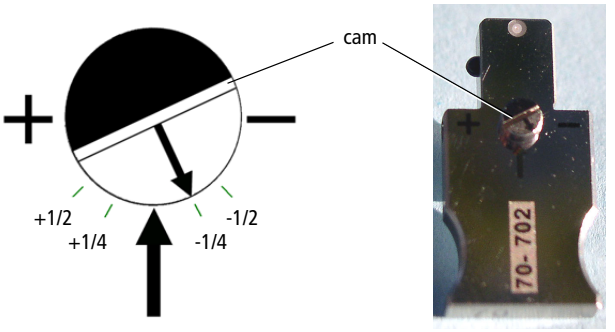
If the satellite drops are not merging properly, you will need to turn the cam on the adjustable nozzle until you find the proper setting for your instrument cuvette.

1. Open the flow cell access door.

2. Turn off the stream and remove the nozzle.

3. Turn the cam until the small arrow is approximately 1/4 of the distance between the large arrow and the negative symbol (-1/4 in Figure 6-8).

Figure 6-8 Adjusting the nozzle



Use the coated screwdriver (part no. 336984) provided with the nozzle to turn the cam.

4. Carefully insert the nozzle, turn on the stream, and observe the satellite drop formation.

NOTICE Make sure there are no bubbles in the flow cell to interfere with drop formation. If you see bubbles, turn off the stream, wait a few seconds, and turn on the stream again.

5. If the satellites are not merging properly, repeat steps 2 and 3, turning the cam in the opposite direction.

Turn the cam until the small arrow is approximately 1/4 the distance between the large arrow and the positive symbol (+1/4 in Figure 6-8).

6 Repeat step 4.

7 If the satellites are still not merging properly, repeat steps 2 and 3, turning the cam until the small arrow is halfway between the large arrow and the negative symbol (-1/2 in Figure 6-8); repeat step 4.

8 If the satellites are still not merging properly, repeat steps 2 and 3, turning the cam until the small arrow is halfway between the large arrow and the positive symbol (+1/2 in Figure 6-8); repeat step 4.

9 If needed, continue adjusting the cam to achieve the required satellite drop pattern.

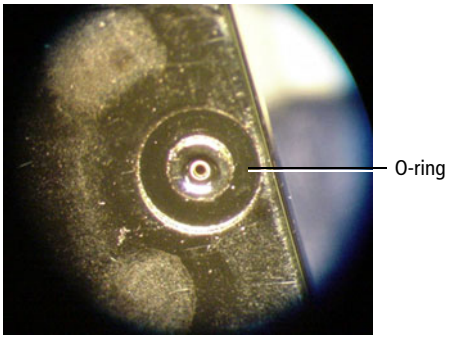
NOTICE The cam adjustment range extends 180° between the positive and negative symbols on the adjustable nozzle. If the large arrow points to the shaded (black) half of the cam, the nozzle adjustment is out of range. For proper nozzle operation, do not adjust the cam outside its adjustment range.

10 Turn on the Sweet Spot when the drop pattern is stable.

Cleaning a Nozzle

Use one of the following methods to clean the nozzle when the stream appears blocked or distorted. To verify that the nozzle is clogged, examine the tip under a microscope. Figure 6-9 shows an example of an unclogged nozzle tip.

Figure 6-9 Magnified view of a nozzle tip



All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle nozzles as if capable of transmitting infection. Wear suitable protective clothing and gloves.

1. Open the flow cell access door and turn off the stream.
2. Remove the nozzle from the cuvette flow cell.

   Turn the nozzle-locking lever 90° to the left. Remove the nozzle by pulling it straight out.
3. Remove and set aside the O-ring.
4. Sonicate the nozzle for approximately 1 minute.

   Sonicate the nozzle in a test tube containing DI water or a mild detergent. Repeat the sonication as needed until the nozzle is clean.
5. Thoroughly dry the nozzle.

Wipe it gently with optical lens paper. Make sure no fibers are left behind.

6 Use the wooden end of a swab to reinstall the O-ring on the nozzle tip.

7 Carefully reinsert the nozzle into the flow cell.

Insert it at a slight downward angle to avoid contact between the O-ring and the cuvette. Push it gently all the way forward without rocking it from side to side. Ensure that the nozzle is in place and the O-ring is seated properly.

8 Turn the nozzle-locking lever clockwise to the 12:00 position.

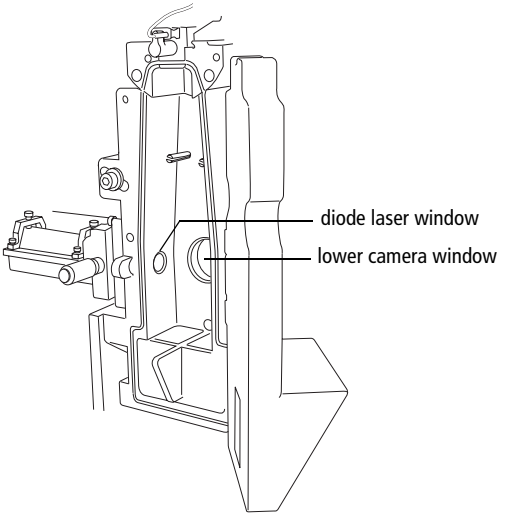
9 Turn on the stream, and make sure it flows through the nozzle properly.

10 Close the flow cell access door.

Note that after reinstalling the nozzle, you might need to change the angle of the sort block to re-center the stream in the aspirator. To do so, loosen the adjustment screws on either side of the deflection plates and rotate the sort block (see Figure 1-11 on page 32). Tighten the screws when the stream is centered in the aspirator. For further assistance, see Troubleshooting the Stream on page 212.

Cleaning the Camera Windows

- Clean the lower camera window and the diode laser window when you have trouble viewing the side streams or you cannot set the drop delay using Accudrop. Wipe the windows with a soft, lint-free cloth soaked with DI water, and then dry the windows.



- Clean the strobe lens and upper camera window when smudges appear in the processed (digitized) image in the Breakoff window, or when dark spots appear to interfere with Sweet Spot monitoring. You might need to clean these components after a clog, or after sheath fluid has leaked or sprayed. Follow the steps in this section to clean the strobe lens and upper camera window.

Before you begin this procedure, block the lasers, turn off the stream, and ensure that the deflection plates are not charged (warning light is not illuminated).

⚠⚠ A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the plate voltage is on. The plates remain energized even when the sort block door is opened.

1 Open the sort block door and remove the deflection plates.

Pull the plates straight out to remove them.

2 Remove the nozzle.

For detailed instructions, see Changing the Nozzle on page 199. (You do not need to remove the lever.)

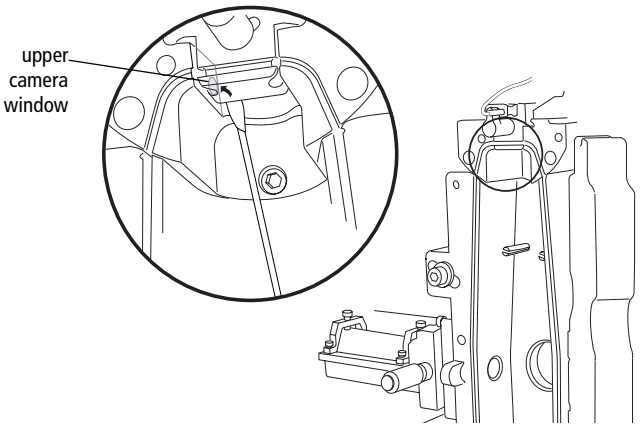
3 Place 1–2 drops of DI water or ethanol on a cotton swab.

4 Right-click the Breakoff window and choose Raw Image.

Visible smudges are more apparent in the raw image view.

5 While viewing the image on the Breakoff window, insert the swab just below the bottom of the cuvette.

The strobe lens and upper camera window are located behind two circular openings on either side of the cuvette. You will see the end of the swab in the Breakoff window when you intercept either opening.



6 Gently wipe the upper camera window, and then the strobe lens (opposite the window) to remove any saline.

7 Repeat with spectral-grade methanol or absolute ethanol until clean.

8 Reinstall the nozzle and deflection plates.

See Changing the Nozzle on page 199 for details.

9 Adjust the plates so there is a gap of approximately 0.28 in. (0.7 cm) between them at the top.

Cleaning Optical Filters

Optical filters should be inspected occasionally and cleaned as necessary. The frequency will depend on how often the filters are handled.

⚠ When cleaning or replacing a filter, handle with care to avoid scratching the surface and to prevent the filter from falling out of the holder. Use cotton swab, optical lens paper, and spectral-grade methanol or absolute ethanol in a dropper bottle (do not use acetone) to clean optical filters.

1 Wrap a triangular section of the lens paper around the cotton end of a cotton swab. Moisten and seal the end with a few drops of alcohol.

2 Holding the cotton swab in a horizontal position, gently rub any spots on the filter surface and wipe clean.

3 Allow the solvent to evaporate, and check the filter surface for streaks.

4 Inspect a quarter-inch–diameter section in the center of the filter for scratches.

Filters are coated with different dielectrics that can get scratched. If you see scratches, replace the filter.

5 Insert the cleaned filter into the octagon or trigon.

Make sure the filters are pushed all the way in.

Purging the Bubble Filter

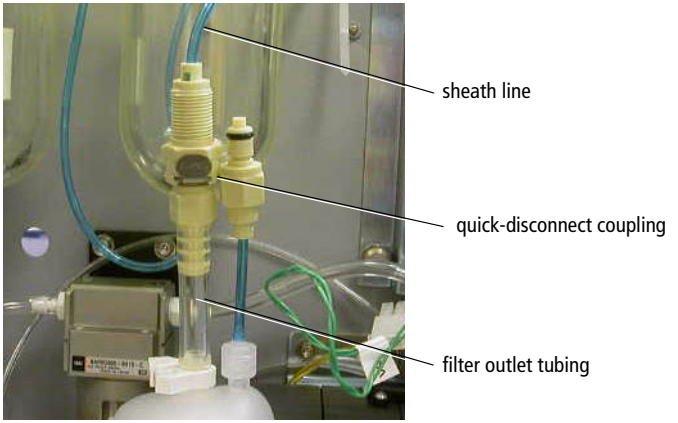
The bubble filter is a standard 0.2-µm fluid filter that helps keep air bubbles and small particles from reaching the flow cell. To maintain the filter you will need to periodically purge the air that accumulates in it.

During normal instrument operation, the outlet tubing at the top of the bubble filter is connected to the sheath line leading to the flow cell. To purge the filter, you need to disconnect the sheath line from the filter outlet tubing and connect the sheath line to the filter purge tubing.

⚠⚠ Moisture in electrical circuitry can result in a short-circuit or present a shock hazard. To prevent fluid from spilling onto electrical components, wrap connectors loosely in paper towels before disconnecting any fluid lines. Use paper towels to absorb the excess fluid.

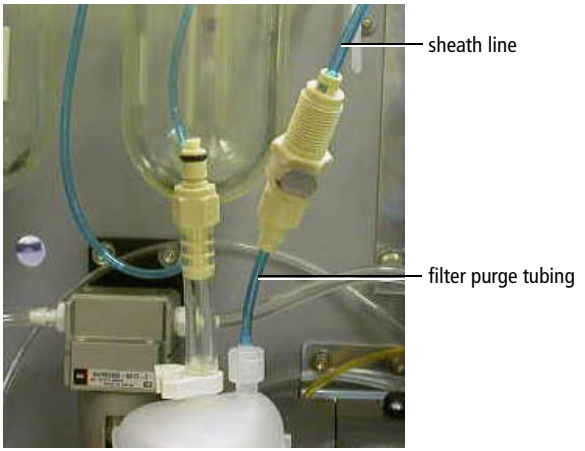
1. Reduce the sheath pressure.
   - Choose Instrument > Sheath Pressure.
   - Take note of the current sheath pressure—you will need to return the value to this setting after the filter purge is complete.
   - Change the pressure to 10 psi, and click OK.
2. Using a paper towel to cover the coupling, disconnect the sheath line from the filter outlet tubing by pressing the tab on the quick-disconnect coupling (Figure 6-10 on page 209).

Figure 6-10 Filter outlet tubing connected to sheath line



3 Connect the sheath line to the filter purge tubing (Figure 6-11).

Figure 6-11 Filter purge tubing connected to sheath line



4 Turn on the stream.

5 Gently tap the bubble filter to ensure that there are no large air bubbles left in the filter.

6 Turn off the stream.

7 Remove the sheath line from the filter purge tubing and reconnect the sheath line to the filter outlet tubing as shown in Figure 6-10.

8 Turn on the stream and ensure that there are no bubbles in the flow cell.

9 Repeat the stream-off and stream-on modes as necessary until all bubbles are removed from the flow cell.

10 Increase the sheath pressure to its original value.

Choose Instrument > Sheath Pressure and input the setting from step 1.

NOTICE If the instrument is run until the sheath supply is depleted, air will accumulate in the bubble filter. Purge the filter as described in Purging the Bubble Filter on page 208. If you are unable to remove all of the air, replace the bubble filter as described in Changing the Bubble Filter on page 197.

7

Troubleshooting

The tips in this section are designed to help you troubleshoot your experiments. Additional troubleshooting information can be found in the *BD FACSDiva Software Reference Manual*.

If additional assistance is required, contact your local BD Biosciences technical support representative. See Technical Assistance on page xi.

Troubleshooting suggestions in this chapter are grouped under the following headings:

- Troubleshooting the Stream on page 212
- Troubleshooting the Breakoff on page 216
- Sorting Troubleshooting on page 217
- Acquisition Troubleshooting on page 222
- Fluidics Troubleshooting on page 229
- Electronics Troubleshooting on page 230

Troubleshooting the Stream

Observation	Possible Causes	Recommended Solutions
Stream not in center of aspirator	Difference in keyed stream position between nozzles	If you have just changed the nozzle, use an Allen wrench to loosen the screws on either side of the sort block. Adjust the angle of the sort block until the stream flows into the center of the waste aspirator, and then tighten the screws. See Figure 1-11 on page 32.
	Nozzle inserted improperly	Turn off the stream. Remove the nozzle and ensure that the O-ring is in place. Reinsert the nozzle at a slight downward angle to prevent loss or movement of the O-ring. Make sure the nozzle is completely seated against the back wall of the cuvette flow cell.
	Clogged or damaged nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. • If debris is visible, clean the nozzle. See Cleaning a Nozzle on page 203. • If the nozzle appears damaged, replace it. See Changing the Nozzle on page 199.

Troubleshooting the Stream (continued)

Observation	Possible Causes	Recommended Solutions
No stream or dripping stream	Nozzle inserted improperly	Turn off the stream. Remove the nozzle. See Changing the Nozzle on page 199 for instructions.
	Clogged or damaged nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. • If debris is visible, clean the nozzle. See Cleaning a Nozzle on page 203. • If the nozzle appears damaged, replace it. See Changing the Nozzle on page 199.
Stream control disabled or no stream when Stream control clicked	Plenum not full	Wait for the plenum to fill.
	Air lock in bubble or fluidics filter	Prime the system with the corresponding fluid. If the control is still disabled, remove the filter, install bypass tubing, and repeat the priming procedure until you see fluid in the line. When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.
	Communication failure between workstation and instrument	Quit the software and restart it.

Troubleshooting the Stream (continued)

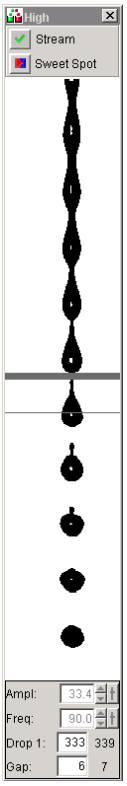
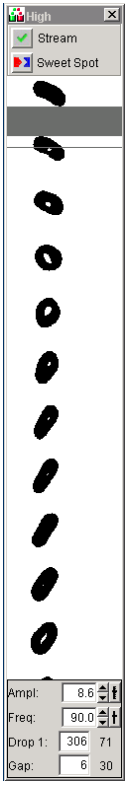
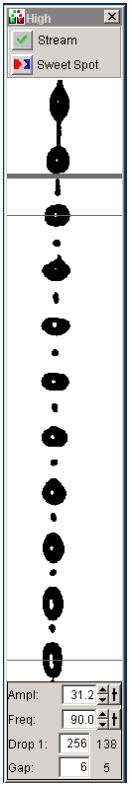
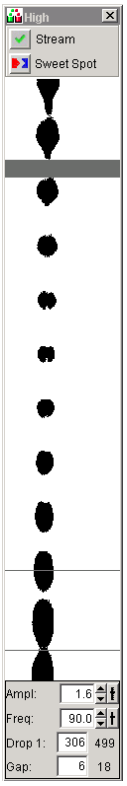
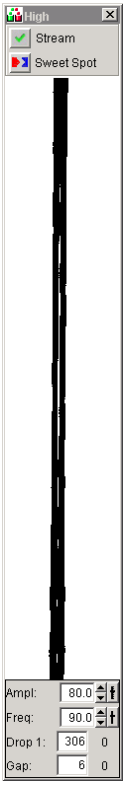
Observation	Possible Causes	Recommended Solutions
No stream when Stream control clicked	Air in bubble filter	Purge the bubble filter. See Purging the Bubble Filter on page 208.
	Sheath container low or empty	Refill the sheath tank. See Refilling Containers on page 109. Note that when the empty tank warning message is not dismissed after 15 minutes, the stream shuts off automatically.
	Air in sheath line	Prime the sheath tank. See Prime After Tank Refill on page 179.
	Air in sheath filter	Purge the sheath filter. See Purging Filters on page 192.
	Dry filter	**1** Install bypass tubing in place of the filter for the affected fluid, and run Prime After Tank Refill. **2** Reinstall the filter and open the bleeder valve to purge the filter. See Purging Filters on page 192.
Fanning around center stream	Nozzle inserted improperly	Reinsert the nozzle. Push it gently all the way forward without rocking it from side to side.
Unstable stream	Debris in flow cell or nozzle	Remove the nozzle and run the stream with no nozzle in place for approximately 10 seconds. (Click the Stream control on, and then off.) Sonicate the nozzle and reinstall it.

Troubleshooting the Stream (continued)

Observation	Possible Causes	Recommended Solutions
Leaking or spraying around nozzle	Defective nozzle O-ring	Replace the O-ring.
	Nozzle inserted improperly	Turn off the stream. Remove the nozzle. See Changing the Nozzle on page 199 for instructions.
	Extra O-ring blocking nozzle	Remove the nozzle and use a cotton swab to clear out the cuvette.
Drop breakoff too long	Bubbles in flow cell	Open the flow cell access door and check for bubbles in the flow cell. If they are visible, turn off the stream, wait a few seconds, and turn on the stream again.
	Attenuation on	Turn off attenuation.
	Amplitude too low	Increase the amplitude until you can see drops. If you need a very high amplitude (>70 volts) to see drops, you might have air bubbles in the flow cell.
	Nozzle inserted improperly	Turn off the stream. Remove the nozzle and ensure that the O-ring is in place. Reinsert the nozzle at a slight downward angle to prevent loss or movement of the O-ring. Make sure the nozzle is completely seated against the back wall of the cuvette flow cell.

Troubleshooting the Breakoff

Use the following examples to help troubleshoot problems with the breakoff image.

	Abnormal Stream Image					
					
Normal stream image	**Possible Causes**	Nozzle inserted improperly	Nozzle inserted improperly or orifice off center	Partial clog	Wet or dirty strobe lens	Attenuation is on at wrong pressure
	Recommended Solutions	Remove the nozzle and reinsert it.	Remove the nozzle, adjust the opening, and reinsert it.	Remove the nozzle, clean it, and then reinsert it.	Clean the lens as described in Cleaning the Camera Windows on page 205.	Turn off attenuation in the Side Stream window.

Sorting Troubleshooting

Observation	Possible Causes	Recommended Solutions
Unstable breakoff while Sweet Spot engaged	Residual ethanol in system	Allow the system to run until the breakoff stabilizes.
	Target Drop 1 value out of range for drop spacing	Use an actual Drop 1 value for the target. Remember to repeat the drop delay setup each time the target value is changed.
	Nozzle clogged or inserted improperly	Turn off the stream. Remove the nozzle and clean it. Reinsert the nozzle at a slight downward angle to prevent loss or movement of the O-ring. Make sure the nozzle is completely seated against the back wall of the cuvette flow cell.
	Dirty strobe lens or upper camera window	Clean the lens and the window as described in Cleaning the Camera Windows on page 205.
	Air lock in bubble or fluidics filter	Prime the system with the corresponding fluid. If the control is still disabled, remove the filter, install bypass tubing, and repeat the priming procedure until you see fluid in the line. When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.
	Debris in flow cell or nozzle	Remove the nozzle and run the stream with no nozzle in place for approximately 10 seconds. (Click the Stream control on, and then off.) Sonicate the nozzle and reinstall it.

Sorting Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Center stream image dim or not visible in Side Stream window	Camera window dirty	Clean the lower camera window. See Cleaning the Camera Windows on page 205.
	Stream not intercepting diode laser	Adjust the micrometer dial on the laser to make the stream intercept as bright as possible. See Figure 1-17 on page 41.
Center stream off center when plate voltage turned on	Voltage Center too low or too high	Adjust the Voltage Center slider to put the center stream back to center.
	Saline spray on deflection plates or in sort block	Clean the deflection plates and the area around them.
Arcing between deflection plates	Plates too close together at the top	Adjust the plates so there is a gap of approximately 0.28 in. (0.7 cm) between them at the top.
	Salt bridge	Clean and dry the deflection plates and the area around and behind the plates.
ACDU sorting failure	Insufficient stream voltage	Increase the voltage for the far left stream.
	Splash shield not installed	Install the splash shield. See Installing the Sorting Hardware on page 163.

Sorting Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No deflection or insufficient deflection	Insufficient voltage	• Increase the plate voltage. • Increase the side-stream voltages using the slider controls.
	Stream-charging wire loose or missing	Verify the stream-charging wire is inserted all the way into the barb.
	Salt bridge	Turn off the stream. Remove the nozzle. See Changing the Nozzle on page 199 for instructions.
	Plates too far apart at top	Adjust the plates so there is a gap of approximately 0.28 in. (0.7 cm) between them at the top.
	Sorting paused because actual Drop 1 value out of range	Wait until the Sweet Spot adjusts the amplitude to achieve the Drop 1 target. If this happens repeatedly during sorting, you could have debris in the nozzle or flow cell. See Troubleshooting the Stream on page 212 for suggestions.

Sorting Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Fanning around center or side streams	Nozzle inserted improperly	Turn off the stream. Remove the nozzle and ensure that the O-ring is in place. Reinsert the nozzle at a slight downward angle to prevent loss or movement of the O-ring. Make sure the nozzle is completely seated against the back wall of the cuvette flow cell.
	Sweet Spot off	Turn on the Sweet Spot.
	Incorrect Sort Precision mode	Verify that the Sort Precision mode is appropriate for your sorting requirements. See Sort Precision Modes on page 75.
	2nd, 3rd, 4th Drop values not optimized	Adjust the 2nd, 3rd, and 4th Drop settings to tighten the center stream and fine-tune the side streams.
	Particles too big for nozzle	Change the nozzle. See Changing the Nozzle on page 199.
Sort button disabled	Current tube pointer not set to current tube	Click to move the current tube pointer in front of the appropriate tube.
Population not listed in Add menu on sort layout	Population defined using Snap-To gate	Redefine the population using another gate type.
	Viewing sort layout for another tube	Open or create a sort layout for the current acquisition tube.
Sort layout counters not updating	Viewing sort layout for another tube	Open or create a sort layout for the current acquisition tube.

Sorting Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
High sort conflict rate	Event rate too high for drop drive frequency	Decrease the event rate.
	Gating conflict	Verify the gating hierarchy.
	Purity Mask too high	Decrease the Purity Mask.
Erratic sort rate	Flow Rate too high	Decrease the Flow Rate.
Unexpected sort results	Incorrect drop delay	Reset the drop delay. See Determining the Drop Delay on page 154.
	Incorrect Sort Precision mode	Verify that the Sort Precision mode is appropriate for your sorting requirements. See Sort Precision Modes on page 75.
	Sweet Spot off	Keep the Sweet Spot on during sorting.
	Drop 1 changed after setting drop delay	Reset the drop delay each time you change the Drop 1 value. See Determining the Drop Delay on page 154.
	Laser delay changed after setting drop delay	Reset the drop delay each time you change the laser delay. See Determining the Drop Delay on page 154.
	Incorrect logic in Population Hierarchy	Verify the gating strategy.

Sorting Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unexpected sort results (continued)	Sorting parent and child populations into two different tubes	If you try to sort a parent and its child population into two tubes, BD FACSDiva software ignores the child events in both tubes. Create a new subset under the parent population consisting of NOT (Child). Sort the child population into one tube and the NOT (Child) population into another tube.

Acquisition Troubleshooting

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Load or Acquire Data	Current tube pointer not set to current tube	Click to move the current tube pointer in front of the appropriate tube.
	Laser shutter engaged	Make sure the flow cell access door is completely closed.
	Laser power off	Turn on the laser power.
	Laser delay set incorrectly	Adjust the laser delay settings. See Instrument Quality Control on page 113.
	Viewing plots for a different tube	Double-click the current tube in the Browser to display the plots for that tube.
	Incorrect population(s) in plot	Right-click the plot and choose Show Populations. Verify that the appropriate populations are displayed.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Acquire Data (continued)	Uncolored events in plot	• Format the plot to display all events. • Assign a color to the population displayed in the plot. • Verify the population drawing order.
	Current instrument configuration different from optical setup	Verify that the instrument optics match the current instrument configuration. See Application Options on page 242.
	No sample in tube	Add sample to tube or install new sample tube.
	Sample not mixed properly	Increase the Sample Agitation rate. See Sample Agitation on page 83.
	Sample line clogged	Perform a sample line backflush. See Sample Line Backflush on page 177. If necessary, change the sample line.
	Threshold not set to correct parameter (usually FSC)	Set the threshold to the correct parameter for your application.
	Multiple Threshold parameters not set correctly	Verify that the correct Boolean logic (And/Or) was used for the Threshold parameters.
	Threshold channel too low or too high	Adjust the Threshold channel. See Adjusting the Voltages and Threshold on page 130.
	Optical filter(s) not completely seated	Make sure the filters are pushed all the way in.
	FSC area setting incorrect	Ensure that the FSC-H matches the FSC-A value.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No fluorescent signal	Current instrument configuration different from optical setup	Verify that the instrument optics match the current instrument configuration.
	Wrong filter installed or filter not completely seated	Make sure the appropriate filter is installed for each fluorochrome; see Application Options on page 242 for suggestions. Make sure the filters are pushed all the way in.
	Laser delay set incorrectly	Adjust the laser delay settings. See Instrument Quality Control on page 113.
Low Area signal	Area scaling too low	Adjust area scaling for the corresponding laser. See Instrument Quality Control on page 113.
Unexpected events in plot	Incorrect logic in Population Hierarchy	Verify the gating strategy.
	Incorrect population(s) in plot	Right-click the plot and choose Show Populations. Verify that the appropriate populations are displayed.
	Incorrect drawing order	Verify that the required population is not hidden by another population. Right-click the plot and choose Order Populations by Count.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Erratic event rate	Sample aggregates	Filter the sample.
	Bulk injection O-ring worn	Contact your BD Biosciences service engineer.
	Sample contaminated	Re-stain the sample, making sure the tube is clean.
	Sheath container low	Fill the sheath container.
Unexpectedly high event rate	Threshold channel too low	Adjust the Threshold channel. See Adjusting the Voltages and Threshold on page 130.
	Sample too concentrated	Dilute the sample.
	Event rate too high	Decrease the Flow Rate in the Acquisition Dashboard.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and turn on the stream again.
Unexpectedly low event rate	Sample not adequately mixed	Increase the Sample Agitation rate. See Sample Agitation on page 83.
	Threshold channel too high	Adjust the Threshold channel. See Adjusting the Voltages and Threshold on page 130.
	Sample too dilute	Concentrate the sample.
	Sample line clogged or kinked	Backflush the sample line. See Sample Line Backflush on page 177. If necessary, change the sample line.
		Look for visible kinks in the line. If kinks are noted, change the sample line. See Changing the Sample Lines on page 194.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unexpectedly low event rate (continued)	Sample line installed incorrectly	Verify the sample line installation. See Changing the Sample Lines on page 194.
	Sample aggregates	Filter the sample.
	Memory full	Compare the processed event rate in BD FACSDiva software with the threshold counter on the instrument. If the software event rate is much lower, quit and then restart the application.
Distorted parameters or high CVs	Instrument settings adjusted incorrectly	Optimize the scatter parameters. See Adjusting the Voltages and Threshold on page 130.
	Flow rate too high	Decrease the Flow Rate in the Acquisition Dashboard.
	Window extension too low	Increase the window extension.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and turn on the stream again.
	Nozzle clogged or dirty	Clean the nozzle as described in Cleaning a Nozzle on page 203.
	Flow cell dirty	Clean the flow cell with a detergent such as Contrad. See Clean Flow Cell on page 178. Let the detergent sit for 5 minutes before turning on the stream.
	Poor sample preparation	Repeat sample preparation.
	Area scaling too low	Verify area scaling. See Running QC Particles on page 116.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Excessive amount of debris in plots	Threshold channel too low	Increase the Threshold channel. See Adjusting the Voltages and Threshold on page 130.
	Dead cells or debris in sample	Examine the sample under a microscope.
	Sample contaminated	Re-stain the sample, making sure the tube is clean.
High electronic abort rate (>10% of system event rate)	Window extension too high	Decrease the window extension.
	Threshold channel too low	Increase the threshold channel.
	Event rate too high	Decrease the Flow Rate in the Acquisition Dashboard.
	Sample aggregated	Filter the sample.
	Sample too concentrated	Dilute the sample.
Fewer events than expected in gated population	Window extension set incorrectly	Adjust the window extension, if needed. Refer to the *BD FACSDiva Software Reference Manual* for information.
	Laser delay set incorrectly	Adjust the laser delay settings. See Instrument Quality Control on page 113.
	Plot zoomed	Unzoom the plot or make the gate bigger.
	Events left out of gate	When drawing a gate, make sure events on the axis are included.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Increasing threshold results in decreased Area signal	Window extension too low	Slightly increase the window extension to maximize Area signal. **NOTICE** Increasing the window extension too much results in more electronic aborts or high CVs.
Area measurement off-scale while the Height measurement is on scale	Area scaling too high	Decrease area scaling to move the Area measurement back on scale. If necessary, adjust area scaling to make the Area measurement match the Height measurement.
Cannot delete from Parameters, Threshold, Compensation, or Ratio tab views	Row not selected	Select the row using the selection button.
	Data already recorded	Create a new tube.

Fluidics Troubleshooting

Observation	Possible Causes	Recommended Solutions
No fluid in line during system prime	Air lock in bubble or fluidics filter	Remove the filter for the corresponding fluid, install bypass tubing, and run Prime After Tank Refill. Repeat the priming procedure until you see fluid in the line. When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.
Long clean fails	Air lock in filter	See previous recommendations.
	Fluid line detached	Verify the fluid line connections on the fluidics cart and on the instrument. Push firmly on each line to ensure it is connected.
Fluidics cart air flow <70 psi	Air leak	Contact your BD Biosciences service engineer.
Fluidics cart air flow >100 psi	Regulator not adjusted properly	Contact your BD Biosciences service engineer.
Fluid leak under fluidics cart or below side door	Condensation from pressure relief valve	This is a normal phenomenon that occurs when water is condensed from room air. Condensation is greater in humid environments. To avoid slipping, check and wipe up the water daily.
	Bleeder valve open	Check and close all bleeder valves. See Purging Filters on page 192.
	Broken fluid line	Contact BD Biosciences.
Fluid leaks at bubble filter ports	Defective filter or loose connection	Replace the bubble filter. See Changing the Bubble Filter on page 197.

Electronics Troubleshooting

Observation	Possible Causes	Recommended Solutions
"Instrument Disconnected" in Instrument window	Instrument power off	Turn on the instrument main power.
	Communication failure between workstation and instrument	• Quit the software and then restart it. • If restarting does not work, reset the instrument electronics: switch off the main power, wait 10 seconds until the system is fully depressurized, and then switch the power back on. • Restart the computer and the instrument.
	Ethernet cable disconnected between workstation and instrument	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address changed	Enter the correct IP address. Call BD Biosciences for assistance.
"Master DAQ Overflow" in Instrument window	Event rate too high	Decrease the event rate or verify the threshold.
	Dirty flow cell	Clean the flow cell. See Clean Flow Cell on page 178.
"Instrument not responding" in Status tab	Unknown	Perform the suggestions for a communication failure, above.

Appendix A

Supplies and Options

This appendix provides a list of supplies and options that are available for the BD FACSAria instrument.

- To order spare parts and consumables from BD Biosciences from within the US, call: (877) 232-8995 or go to bdbiosciences.com.

  In other countries, contact your local BD Biosciences representative.

- To order instrument options, contact your sales representative.

This information is correct at the time of publication; for up-to-date information refer to our website (bdbiosciences.com).

Instrument Supplies

Optical Components

The following filters and mirrors are mounted on the BD FACSAria instrument. Use these part numbers if you need to order any replacement components. See Changing Filters on page 247 for instructions.

Detector Array (Laser)	PMT	LP Mirror BP Filter	Replacement Part No.	Intended Dye
Octagon (488-nm blue laser)	A	735	343787	
		780/60	343788	PE-Cy7
	B	655	343789	
		695/40	343790	PerCP-Cy5.5 or PI
		675/20	343791	PerCP
	C	610	640879	
		616/23	640880	PE-Texas Red
	D	556	343794	
		585/42	343796	PE or PI
	E	502	343797	
		530/30	343798	FITC
	F	488/10	343799	SSC
		1.0 ND filter	53-10057-01	
Trigon (633-nm red laser)	A	735	343787	
		780/60	343788	APC-Cy7
	B	660/20	343800	APC

The following filters and mirrors are provided with the violet-laser option.

Detector Array (Laser)	PMT	LP Mirror BP Filter	Replacement Part No.	Intended Dye
Trigon (407-nm violet laser)	A	502	343797	
		530/30	343798	Alexa Fluor 430
	B	450/40	343801	Cascade blue, Pacific blue, Hoechst, DAPI, Alexa Fluor 405

The FSC photodiode is provided with a 1.0 ND filter. Other filters are available as options. Use the following part numbers to replace the filter.

Detector	ND Filter	Replacement Part No.
FSC photodiode	1.0	33710807
	0.5	33710407
	1.5	338651
	2.0	338652

To order a filter holder, use the following part numbers.

Holder	Part No.
Mirror holder	336103
Mirror retainer	345662
Mirror backup spring	344979
Filter holder	336102
Filter retainer	333830

Accessory Kit

The instrument is shipped with an accessory kit containing the following items. Use these part numbers if you need to order any replacements.

Item	Part No.
1-mL microtube holder	333457
12 x 75-mm test-tube holder	333456
15-mL centrifuge-tube holder	333430
Four-way 1-mL collection tube holder	345898
Four-way 12 x 75-mm collection tube holder	333532
Two-way 12 x 75-mm collection tube holder	334904
Two-way 15-mL collection tube holder	345896
O-ring for collection-tube holder	337897
Adaptor tray for microscope slides	335630
ACDU splash shield	334909

Item	Part No.
Spare nozzles: • 70 micron • 100 micron	 342908 342909
Nozzle locking lever (spring and plunger included)	337579
Nozzle O-rings (bag of 30; replacements sold individually)	333084 (each)
Magnifying glass	33759907
Sample injection tubing (three 12-in. lengths)	335598
HPLC valve tubing (three 7-in. lengths)	335599
Ferrule (1)	335108
Ferrule-removal tool	335690
Bubble filter (1)	33712707
Fluidics cart tubing replacement kit: • 1/4 x 1/8-in. sheath tubing (96-in. length, blue) • 1/4 x 1/8-in. waste tubing (288-in. length, orange) • 3/8 x 1/4-in. air tubing (96-in. length, clear) • 3/8 x 1/4-in. waste tubing (96-in. length, yellow) • Fluidics cart power cord	33545707
Fluid filter assembly (Pall SuporCap 50, 0.2 µm)	33566307
Bypass tubing assembly (tubing and quick-disconnect fittings)	33576007
Disposable waste tank caps (pack of 12)	33885407
Cap for 5-L container (5)	335916
Cap for 10-L container (5)	335917

Item	Part No.
Power cords: • Main power cord • Cordset for continental Europe • Cordset for UK • Cordset for Australia/Asia	 • 333694 • 334140 • 334141 • 334175
Allen wrench set	98-10004-00

Other Replacement Parts

The following items are not included in the accessory kit, but you can use the indicated part numbers to order spare or replacement parts.

Item	Part No.
Autoclavable 10-L container	340261
Auxiliary 5-L container	333504
Sheath sensor probe (5-level)	334914
Waste sensor probe (6-level)	334915
Auxiliary sensor probe (1-level)	334911
Air filter for plenum cabinet (side door)	334351
Air filter for ACDU cabinet	334821 (set of 3)
ULPA filter and tubing replacement kit (set of 3, for use with the AMO)	334822

Laser Specifications

The following class 3B lasers are mounted on the BD FACSAria instrument.

	Manufacturer	Model	Wavelength (nm)	Power (mW)
Excitation Lasers	Coherent	Sapphire 488-20	488	13
	JDS Uniphase	1144-P	633	11
	Point Source	IFlex 2000-P-1-405-0.65-10	407	10
Illumination Laser	StokerYale/Lasiris	SNF-701L-660-35-1 deg single-line laser	660	35

Consumables

Instrument Setup Particles

Particle	Supplier	Catalog No.
BD Calibrite™ Beads • Two-color kit (unlabeled, FITC, PE) • Three-color kit (unlabeled, FITC, PE, PerCP) • PerCP beads • PerCP-Cy5.5 beads • APC beads	BD Biosciences	 • 349502 • 340486 • 340497 • 345036 • 340487
Fluoresbrite™ Yellow-Green 2-µm beads (for the 407-nm laser)	Polysciences Inc. (800) 523-2575	18604
SPHERO™ Rainbow Calibration Particles, 3.0–3.4 µm	BD Biosciences	• 559123 (8 peaks) • 556286 • 556291 (brightest peak in 556286)
BD FACS Accudrop beads	BD Biosciences	345249

Reagents

Reagent	Supplier	Catalog No.
BD FACSFlow sheath fluid	BD Biosciences	340398 (US and Latin America) 342003 (other countries)
BD FACSRinse detergent	BD Biosciences	340346
BD FACSClean solution	BD Biosciences	340345
Ethanol	Various	–
Chlorine bleach (5% sodium hypochlorite)	Clorox® or other major supplier (to ensure that the bleach is at the correct concentration and free of particulate matter)	–
Monoclonal antibodies	BD Biosciences	–[a]
Dyes and fluorochromes	Molecular Probes (800) 438-2209 Sigma (800) 325-3010	–
BD FACS™ lysing solution[b]	BD Biosciences	349202

a. Refer to the BD Biosciences Immunocytometry Products Catalog or the BD Biosciences website (bdbiosciences.com).
b. US Patent Nos. 4,654,312; 4,902,613; 5,098,849

Labware

Item	Supplier	Catalog No.
1-mL microtubes	Bio-Rad Laboratories (800) 424-6723	223-9391 (1,000 per box)
5-mL polystyrene test tubes, 12 x 75-mm (BD Falcon™)	BD Biosciences	
• uncapped, 125 per bag		• 352052
• capped, 125 per bag		• 352054
• capped, 25 per bag		• 352058
• with cell-strainer cap, 25 per bag		• 352235
15-mL conical centrifuge tubes (BD Falcon)	BD Biosciences	
• Polypropylene, 50/bag		• 352196
• Polypropylene, 125/bag		• 352096
• Polypropylene, 50/rack		• 352097
• Polystyrene, 125/bag		• 352095
• Polystyrene, 50/rack		• 352099

Instrument Options

Your BD FACSAria instrument can be upgraded with the following options. Contact your sales representative for more information.

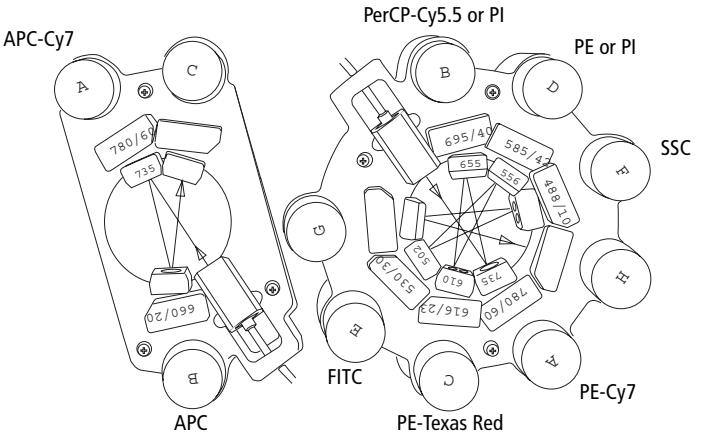
Third-laser option	The third-laser option upgrades your instrument with a violet laser, a trigon, and two PMTs for greater flexibility in analysis and sorting.
Optical upgrade	Optical upgrades are available for two- and three-laser systems to maximize the detection capabilities of the instrument. Each upgrade includes detectors and electronics to detect three or four additional colors, respectively.
Aerosol Management Option (AMO)	The AMO helps contain aerosols by evacuating the sort collection chamber during sorting. The option includes a vacuum source attached to an ultra-low penetrating air (ULPA) filter.
Temperature-control option	This option can be used to control the temperature of sorted samples using a recirculating water bath and metal collection tube holders.
Automated cell deposition unit (ACDU)	The ACDU option provides hardware that allows you to sort into multiwell plates and onto slides.

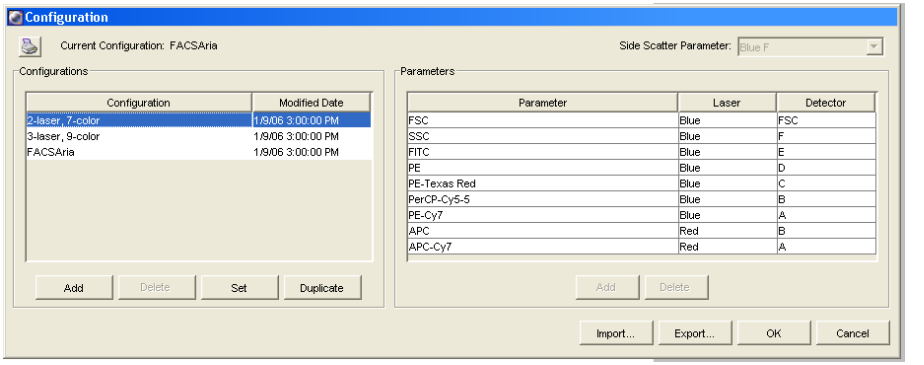
Application Options

The octagon and trigon detector arrays in the BD FACSAria instrument allow many different combinations of mirrors and filters. The following illustrations show how the collection optics are configured for a two- and three-laser system.

Two-Laser System

This is the standard configuration for a two-laser system.

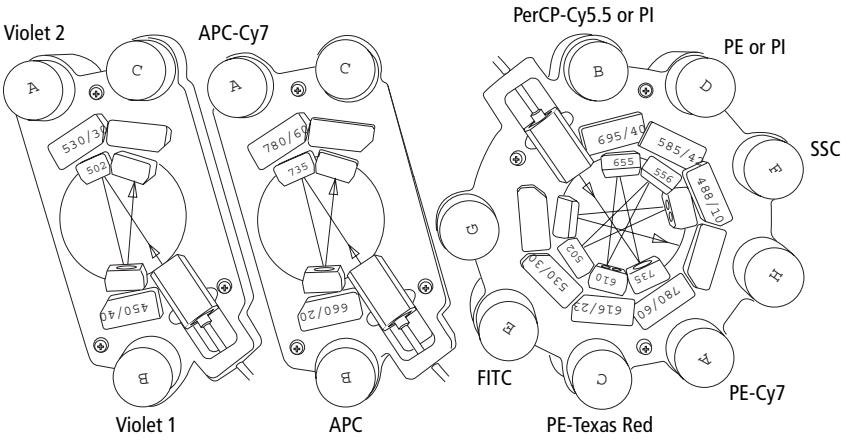


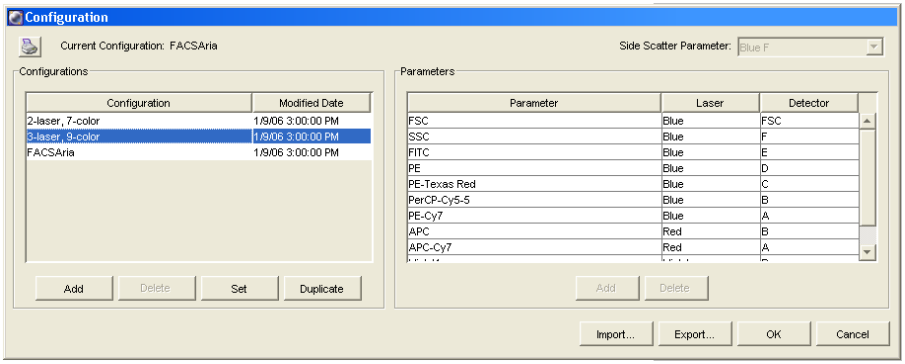


☑ **Tip** To record the PI parameter from Blue D or Blue B, create a custom configuration, or use the FACSAria configuration.

Three-Laser System

This is the standard configuration for a three-laser system.

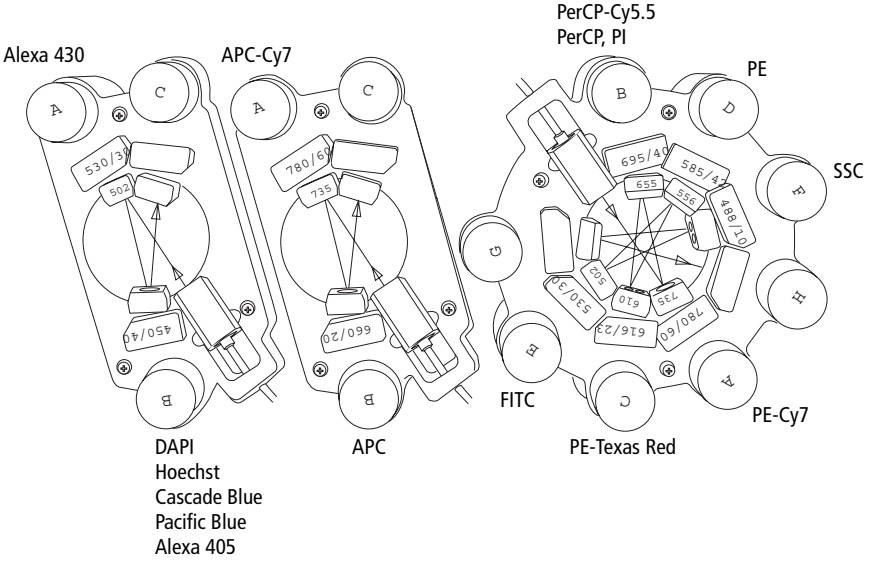


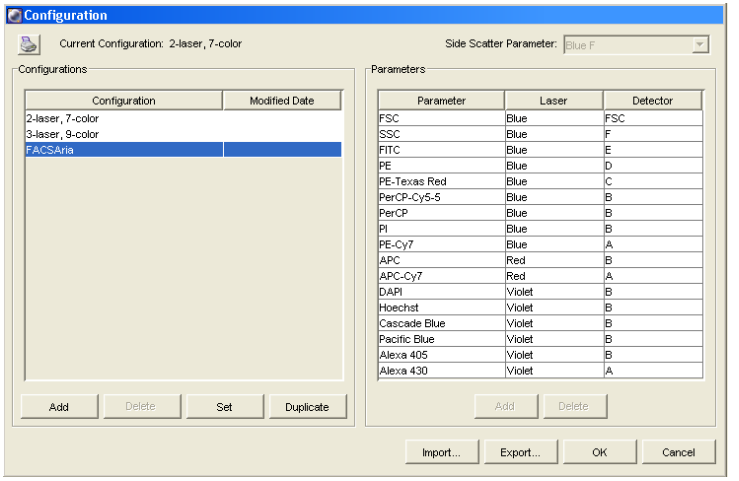


☑ **Tip** To record the PI parameter from Blue D or Blue B, or to include the fluorochrome names for the Violet parameters, create a custom configuration, or use the FACSAria configuration.

FACSAria Configuration

The FACSAria configuration lists multiple fluorochromes for each detector, thus should be applicable for most assays. To ensure that your experiment's parameters match those in the current instrument configuration, BD Biosciences recommends that you always record data with the same configuration.





NOTICE For important information about setting up filters for this configuration, see Changing Filters on page 247.

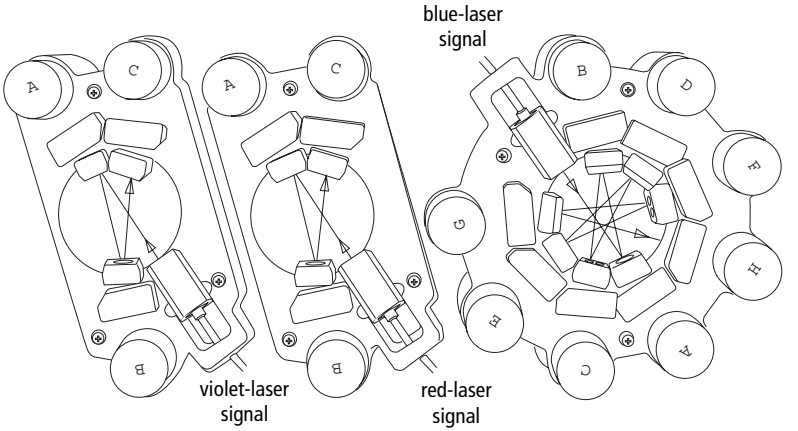
Defining a Custom Configuration

BD FACSDiva software is provided with default configurations specific to your instrument; additional custom configurations can be defined. Refer to the *BD FACSDiva Software Reference Manual* for instructions. To switch between predefined configurations, select the appropriate configuration name, and click Set Configuration.

☑ **Tip** Create one configuration that lists all the fluorochromes used in your laboratory so the same configuration can be used for all experiments. Changing instrument configurations within the same experiment can generate errors. Use the worksheet on page 246 to plan or record a custom configuration.

☑ **Tip** To ensure that an experiment contains the correct parameters, set the configuration you want to use *before* creating a new experiment.

Configuration Name: _____



Detector	Fluorochrome	LP Mirror	BP Filter
Blue ___			
Blue ___			
Blue ___			
Blue ___			
Blue ___			
Blue ___			
Blue ___			
Blue ___			
Red ___			
Red ___			
Red ___			
Red ___			
Violet ___			
Violet ___			
Violet ___			
Violet ___			

Changing Filters

One extra filter is provided in the accessory kit. When you use PerCP rather than PerCP-Cy5.5 in the Blue B position, replace the 695/40 filter with the 675/20 filter to decrease the amount of spectral overlap.

If you want to install a custom filter or dichroic, the filter should comply with the following specifications.

Table A-1 BD FACSAria filter specifications

Filter Characteristic	Dichroic LP Filters	BP Filters
Diameter	0.622 ±0.003 in.	1.00 ±0.010 in.
Thickness	0.125 ±0.005 in.	0.12–0.35 in.
Minimum clear aperture	0.562 in.	0.85 in.
Incident angle	11 ±1°	0°

For the longpass filters, the surface that faces into the center of the octagon/trigon should be coated directly on its surface, not between two or more pieces of glass. The coating should transmit >70% of the wavelength range you want the filter to transmit, with a minimum transmission of >50%, and it should reflect >90% of the wavelength range you want the filter to reflect.

The opposite surface (facing away from the center) should be coated with an anti-reflective coating with a minimum reflection of ≤1% of the wavelength range you want the filter to reflect.

Note that filters must be installed in front of each PMT to block unwanted laser light. For the dichroic, carefully assembly the glass filter in the holder using the retaining spring. Assemble the filter with the arrow pointing toward the center of the octagon or trigon. The angle of the dichroic is critical to achieving optimal results.

Note that any time you modify a detector array, you will need to create a new instrument configuration to identify which PMT (identified by laser color and letter) will detect the emitted light. See Defining a Custom Configuration on page 245 for instructions.

Removing/Installing the FSC ND Filter

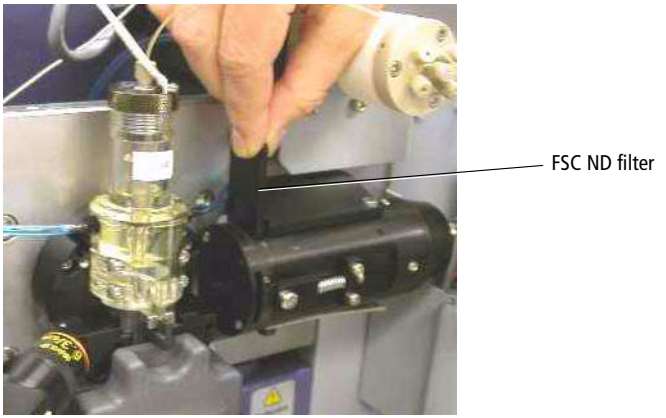
For applications involving large particles where events appear off scale on the FSC axis with a gain of zero, keep the FSC ND filter in place to decrease FSC signal and keep the events on scale. For applications involving small particles (eg, bacteria or platelets), you might need to remove the FSC ND filter as follows.

1 Open the flow cell access door.

2 Locate and pull out the FSC ND filter (Figure A-1).

The filter is installed at the left end of the FSC detector block, just to the right of the flow cell. To remove the filter, pull it out of the slot.

Figure A-1 Removing the FSC ND filter



3 To reinstall the filter, slide it, filter-end down, into the slot.

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